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On the structure
of
Taenia confusa WARD.

By

Michael F. Guyer, A. M.

With one Plate.

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The specimens representing the above named form were obtained through a Lincoln physician; the first one, in 1894, and the second a year later. On account of the external appearance, which is noticeably different from that of the two previously known human Taeniae, *Taenia saginata* and *Taenia solium*, they were set aside for a more careful examination at some future time. The generally peculiar appearance of the body, the dissimilarity in internal structure, and the extremely modified head preclude all possibility of classifying them with either of the common forms. In view of these differences the name *Taenia confusa* was proposed by Dr. Henry B. Ward, who first recognised the specific rank of the form. He briefly mentioned the same in the report of the Zoologist (Ward, 1896, p. 258) and later, in the report for 1896 (Ward, 1897, p. 180—182) gives a fuller general statement in regard to it. The only other literature which refers to the new species is the report of the preliminary announcement made before the Nebraska State Medical Society (Ward, 1896 a). A systematic study of the form was taken up by me in Sept. 1895 and has been carried on in connection with my studies on other forms of Taenia to the present date [June 1897] [1].

The anatomy has been worked out about as thoroughly as the material, which was poorly preserved for histological purposes, would permit. In the following description of the structure of the new worm no great amount of histological detail is given for the reason already stated[1]. Two specimens only, were obtained; the one entire and the other, unfortunately, lacking the head. The last mentioned has been largely used up in making sections, the other remains intact in the private collection of Dr. HENRY B. WARD.

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[1] A recent note referring to this species and to this paper is Ward, 1897 a.

Because of the ease with which specimens of *Taenia saginata* may be secured for study, and also on account of the more abundant supply of literature describing this species, the new form has been compared principally with it although in many such as the general delicacy of structure, the small head, and the branching of the uterus, there is more of a similarity to *Taenia solium*. In the minute details of structure, however, it resembles neither.

General structure. In general the form is from 5 to 8 m long, consisting of a very small head and between 700 and 800 proglottids. The proglottids are, with very few exceptions, longer than broad (Fig. 1); this being especially characteristic of the terminal ones (Fig. 1 *f*), which measure in some cases, as much as 35 mm long by only 4 or 5 mm broad. At no time do they have the peculiar pumpkin-seed shape, so characteristic of the terminal proglottids of *T. saginata*. The uterus, in the ripe proglottids (Fig. 2) consists of a median stem with from 14 to 18 irregularly branching offshoots, which in general appearance often resemble somewhat the branches as seen in *T. solium*. The sexually mature segments (Fig. 1 *c*) are longer than broad and are characterised particularly by the long kidney-shaped lobes of the ovary (Fig. 3 *o*). The whole body is in general much thinner and more fragile than that of *T. saginata*. The eggs (Fig. 11) are oval in shape, 39 μ long by 30 μ wide, of a white color and without a pyriform apparatus.

In the following detailed account, the description of the internal

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1) To Dr. HENRY B. WARD, my highly esteemed instructor, I am greatly indebted for his kindly aid and valuable suggestions. Not only has he supplied me with literature from his own library, but also, with very valuable material for study and comparison from his private collection, to which, in fact, the specimens under discussion belong. His wide acquaintance with zoological literature, and his familiarity with the special methods so essential to a successful pursuit of this kind of research work, have been of inestimable service to me.

structure is based mainly upon observation made on the headless specimen.

External characteristics. The total length of the headless worm is 8.5 m; of the other 5.2 m. Beyond the noticeably long and narrow structure of the proglottids the body does not differ much in general appearance from the ordinary forms. The genital pores are rarely very prominent except in the segments immediately succeeding those sexually mature; as regards the general arrangement in the successive proglottids, there is no anomaly, the pores being arranged alternating irregularly in the usual manner. The body is of slight structure with much less powerful musculature than *T. saginata* possesses, and is incased in a very delicate thin cuticula. The extreme length of some of the segments is the first thing to strike the attention as being unusual and upon measurement it is found that, in the larger worm, at only one point in the chain, just anterior to the sexually mature proglottids, are they wider than long. Here they measure on an average about 3 mm wide by 2.1 mm long, this excess of width over length being present throughout about 80 proglottids. In the smaller one, the predominance of length over breadth although still considerable, is not so marked, and many proglottids measure somewhat wider than long. The general proportions of this worm may be seen from the second table below. In this case, however, most of the segments that are shorter than broad, show by their slightly wrinkled appearance that they are more or less contracted lengthwise.

The following measurements will give some idea of the relative size of different parts of the first worm:

Posterior proglottids . .	35	mm	long	by	3.5 to 4	mm wide
About 1 m further forward	26	„	„	„	5	„
About the middle . . .	15	„	„	„	4.5	„
Showing first branch of uterus	5	„	„	„	4.5	„
Sexually mature	4.5	„	„	„	3.5	„
Near the anterior end . .	1 to 1.2	„	„	„	0.8 to 1 mm	„
The smallest present . .	0.9	„	„	„	0.6	„

From the above data it will be seen that the proglottids are broadest somewhat back of the middle of the worm, and that from the smallness of the anterior segments there could not have been a very great number with the missing head. The whole number of proglottids present is 762.

The second specimen is the one which bears the head[1]). The total length is 5.2 m and the whole number of proglottids, about 775. The proglottids extend quite up to the head and no special, unsegmented neck region is present. The first few are broader than long but change after about the twelfth. The following table gives the measurements of the various parts of the body.

Head	to	50	cm,	number	of	proglottids	290,	the	last	3.2	mm	long	by	4.6	wide
50	,,	100	,,	,,	,,	,,	142,	,,	,,	6	,,	,,	,,	7	,,
100	,,	150	,,	,,	,,	,,	86,	,,	,,	7	,,	,,	,,	8.2	,,
150	,,	200	,,	,,	,,	,,	68,	,,	,,	10	,,	,,	,,	9	,,
200	,,	250	,,	,,	,,	,,	50,	,,	,,	10	,,	,,	,,	10	,,
250	,,	300	,,	,,	,,	,,	47,	,,	,,	13.5	,,	,,	,,	7.8	,,
300	,,	350	,,	,,	,,	,,	36,	,,	,,	15	,,	,,	,,	7	,,
350	,,	400	,,	,,	,,	,,	31,	,,	,,	17	,,	,,	,,	5.4	,,
400	,,	450	,,	,,	,,	,,	20,	,,	,,	29	,,	,,	,,	5	,,
450	,,	488	,,	,,	,,	,,	13,	,,	,,	27	,,	,,	,,	4.8	,,

In general contour the proglottids have somewhat the shape of a flattened cylinder, with the exception of some near the anterior end, and the ones immediately following the sexually mature. The former in many cases show considerable tendency to taper toward the head, and in the later, where the branching of the uterus is well

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1) A description of this structure has been purposely omitted for reasons given elsewhere (WARD, 1897a) from which the following remarks are quoted:

"The head which was attached to the one specimen of *T. confusa* was cut off and after having been stained was mounted in balsam in the ordinary manner. There was, furthermore, a label accompanying the specimen throughout the entire series of transfers and it is difficult for me to believe that it could have been confused with any other specimen, especially since to my knowledge there were no other tapeworms on the table at the same time. . . . It has not escaped any who have examined the figure and description that there exists between the head as described and that of ***Dipylidium*** a great similarity. . . However, the general form appeared to us remarkable on first study, and I am able to say positively, from having studied the head under a lens when still attached to the entire chain, that the head of *T. confusa* was remarkably small, approximately of this same size and shape, and that there was within the head and beneath the apex, a dark object corresponding to the general position of the inverted rostellum with hooks, which was figured from the specimen as mounted and now in my possession. . . . . But since a confusion may possibly have arisen and since I do not wish to prejudice the work of my student by allowing him to incorporate in it any doubtful particulars, I have had him complete his paper omitting any reference to the structure of the head."

under way, the genital pore becomes quite prominent so that the outline takes on a somewhat pentagonal form (Fig. 1 *d*). The proglottids following these begin to lengthen rapidly and broaden gradually until the extreme breadth is attained; in this state they are more or less quadrate in shape (Fig. 1 *e*). The terminal or ripe proglottids do not show in any case the pumpkin-seed form so characteristic of *T. saginata*, and are very slightly smaller at the anterior end, the tapering being almost imperceptible.

The extreme posterior end flares out more or less and forms a broad base for the attachment of the next succeeding segment (Fig. 1 *f*). One striking thing in the ripe proglottid is the prominence of the vagina which persists from its first formation throughout the entire chain. This, however, is not characteristic for this form only but occurs also in *T. saginata* in a less marked degree.

The cuticula. The cuticula is very delicate as compared with of *T. saginata* and can scarcely be detected under the low power of the microscope. It splits or cracks rather easily and is generally thrown into slight folds or wrinkles. On cross section, the body shows a much more irregular or wavy outline than that of *T. saginata*, due doubtless, to the thin cuticula. The cuticula varies slightly in thickness in different proglottids and in various parts of the same proglottid, being somewhat heavier in the posterior ones and very slightly thicker in the neighborhood of the pore. On the dorsal side of a sexually mature proglottid of *T. saginata* it measures 10.5 μ in thickness and correspondingly in *T. confusa* 4.5 μ, in the latter it appears to be homogeneous and in cross section no vertical striations are visible. Nothing resembling cilia or processes of any kind are found on the exterior.

Just beneath the cuticula and seemingly almost a part of it, is a double layer of very minute fibres lying at right angles to each other. The layers are extremely thin and are best seen in frontal section. The transverse fibres are outermost. In neither case is a solid sheath formed, small interspaces remaining between the fibres.

The sub-cuticular layer. Directly under the cuticula, or perhaps more properly, the above mentioned systems of fibres is seen a border or fringe of more or less spindle-shaped cells, at right angles for the most part to the surface. There is a greater or less quantity of intercellular substance present which prevents one from making a very definite study of the cells. They all have prominent nuclei which seem to lie at about the center while one end of the cell lies against the cuticula and, in many cases, the other is seen to be in direct continuity with the single wavy muscle fibres which extend down toward the middle of the proglottid. In most cases, these fibres pass on directly through the band of transverse muscle fibres, but in some instances they seem to run into, and turning, becomes a part of the same.

The body parenchyma. The mass of the body within the cuticula, as in all Cestodes, is made up largely of parenchymatous tissue, which, inasmuch as it differs in no way from that of the ordinary forms, will require no particular description. The whole mass is divided by the transverse and longitudinal muscle fibres, according to the usual manner, into a cortical and middle layer. The middle includes nearly all the organs of the proglottid, and the outer layer, many muscle fibres and calcareous bodies so common to *T. saginata.* The latter, however, are not so plentiful nor so large in this form, measuring only up to 11 μ in diameter. It was suggested that the smaller size might be due to some particular stain used which had partially disintegrated the bodies, but inasmuch as I have used a great variety of stains and find little if any change in the size or structure of these bodies in different preparations, I think the smallness of size can hardly be attributed to the action of the stains.

Muscular system. As has been mentioned the muscular system is much less powerful than that of *T. saginata*; but all three systems of muscles, viz. longitudinal, transverse and sagittal, are present (Fig. 4 *l.m*, *t.m*, *s.m*). The layer of longitudinal muscles is much the thickest of the three and the individual muscle fibres the largest.

They are of especially large size toward the inner edge of the cortical layer and show a tendency to run in bundles; numbers of these bundles lying side by side give the section a marked streaked appearance. In most cases the bundles do not run in exactly straight lines but take a more or less wavy course. Moreover they do not stop at the ends of the proglottids and become attached to the walls, but unquestionably, run on directly from one proglottid to another (Fig. 5 *l.m*). This, then, is very different from the structure found in *T. saginata*, which, according to Leuckart (1886, p. 293) has certain spindle-shaped cells lying between the end of some of the longitudinal fibres in the successive proglottids, connecting them through the isthmus or neck; the fibres themselves always terminating at the end of the proglottid. In *T. confusa* no cells answering to such a description were found. The isthmus contains in addition to the longitudinal fibres, some parenchyma and usually a few transverse and sagittal fibres. This point was verified in sagittal, frontal and transverse sections of proglottids in different stages of development. Some of the fibres near the surface it is true attach to the ordinary spindle cells lying next to the cuticula, where it dips down slightly between the proglottids, but the main mass extends on through into the next segment.

The connection between the various proglottids seems to be the most firm at the sides near the inner edges of the longitudinal excretory canal, these points often being tied together by the longitudinal muscles fibres when all the remaining tissues are free. The proglottids do not in all cases separate along the line marked out on the exterior of the worm, but not infrequently break at the cross excretory canal, which in this form lies very near the posterior edge of the proglottids (Fig. 5 *t.e.c*). This fact was noticed many times in pulling the proglottids apart.

The transverse muscles do not show so much of a tendency to run in bundles as the longitudinal, and are in general smaller. They form two plates (Fig. 4 *t.m*) between which the sexual organs are located. Under the low power they have the appearance of a continuous band but under the higher power they are found to spread at the ends near the edges of the proglottid and for the most part attached to its walls. In the neighborhood of the pore especially their spreading and attachments become very plain. The large central plug in the pore is well supplied with them. Occasionally they may be seen entirely outside the plate and lying in the cortical layer.

The sagittal fibres (Fig. 4 and 5 *s.m*) cross the proglottids from dorsal to ventral surfaces and are scarcely visible under the low power. Those lying in the middle portion of the proglottid have the appearance of connecting the two transverse muscles plates but the higher powers of the microscope show that in reality they pass on, for the most part into the cortical layer. It is due largely to the direction of these fibres that the ends of the transverse plates seem to unite or bend around into one another. These fibres are the smallest of the three kinds mentioned and show little tendency to run in bundles. Often they may be traced across the middle field, through the transverse bands and on into the cortical layer, where in some cases, they attach to the spindle-shaped cells which abut on the cuticula. Near the center of the proglottid they seem to be somewhat more numerous and are crowded together at intervals into band-like masses, appearing to form sheath-like structures around the various internal organs. They persist in the neighborhood of the pore but seem to undergo no modification in structure.

Excretory system. The excretory system does not differ materially from that of *T. saginata.* In some of the very anterior proglottids it is seen at four longitudinal vessels but these soon merge into two, one on either side, which extend through the remainder of the body (Fig. 3 *l.e.c*). The canals are of larger size and near the posterior edge of each segment are joined by a cross vessel (Fig. 3 *t.e.c*). They pass ventral to the genital ducts and between the general inner field and the longitudinal nerves.

Nervous system. On account of the condition of the material, which was not suitable for working out histological detail, but little can be said of the nervous system beyond its location and cross structure. Two main branches extend throughout the body along the outer edge of the large longitudinal excretory canal (Fig. 3 *l.n.s*). They do not exist as single cords, except at the very anterior end but run for the most part in strands of three and occasionally five (Figs. 4 - 6 *l.n.s*). Where three are present the middle one is usually considerably the largest. The strands are for the most part more or less oval in cross section but may spread out until they are almost band-like in appearance. In the neighborhood of the genital ducts they separate, some going to one, and some to the other side of the same, commonly two pass dorsal and one ventral to the ducts. The strands seem to have no distinct sheath of their own, but a sort of surrounding case is formed by the muscles fibres. All are traversed and divided up into minute facets by a connective tissue network. They are almost invariably larger and better developed on the same side with the genital pore.

Sexual organs. In this form as in all other Cestodes, the sexual organs are well developed. They do not differ essentially in their general plan of structure from those of *T. solium* or *T. saginata* but show many minor variations.

Any one of the several more important of these would be almost sufficient alone to show the specific rank of the new form. The proglottids showing full development of the sexual organs measure 4 mm to 4.5 mm long by 3.5 mm wide in the headless worm, and about 4 mm long by 4.5 mm wide in the other; in the first case being longer than broad and in the second somewhat wider than long. In these both male and female systems are well developed, and by means of sections their structure and relationship may readily be made out. As is the case in kindred forms the male organs occupy largely one surface of the body and thus provide means for distinguishing dorsal and ventral side. Following the well established precedent, the side bearing the testes (Fig. 4 *t*) is designated as dorsal.

Male System.

Testes. The testes (Fig. 3 *t*) are present as numerous more or less round bodies distributed throughout the entire upper field of the segment between the excretory vessels, with the exception of a limited area just anterior to the ovarian lobes. They are more abundant toward the borders and anterior end of the inner field. They seem to differ little from those of *T. saginata* except for their smaller size (0.089 to 0.096 mm in diameter). No vasa efferentia are visible but this fact is due probably to the poor histological condition of the material.

Vas deferens. This organ (Fig. 3 and 4 *v.d*) is present as a greatly coiled tube which extends from the genital pore in a line at right angle to the antero-posterior axis of the proglottid, and ends near the center of the segment. The coiling is very pronounced and complicated, and occupies a space from 0.13 to 0.16 mm wide. This area has a sheath-like boundary formed principally of surrounding muscle fibres. The tube itself measures about 0.045 mm in diameter but becomes smaller before it enters the cirrus pouch where it again springs abruptly to a greater diameter forming a special enlargement for storing the innumerable spermatozoa with which it is filled. There are usually from one to three of these bulb-like swellings, which form a sort of Vesicula seminalis.

Vesicula seminalis. This structure (Fig. 6 *v.s*) seems to be modified in no way particularly from the remainder of the Vas deferens, except for the enlargement of the cavity. The diameter of the enlargement varies somewhat in different proglottids, and slightly in the different bulbs, where more than one are present. In general the diameter is about 49.7 μ. The increase in size seems to be principally dorso-ventral, consequently the bulbs can be seen in transverse sections to the best advantage. They are present in all the transverse sections through the pore but in several series of frontal sections they appeared to be lacking until their presence was proven by means of measurements. In a few cases there seemed to be no trace of them in the frontal sections. Occasionally, certain modifications are met with in which the round bulbs show a tendency to merge into one another and form a long vesicle.

Cirrus and cirrus pouch. The cirrus (Figs. 6 and 7 *c*) is simply the modified end of the Vas deferens which, after it reaches the cirrus pouch, becomes much straighter, of less diameter (29 μ) and shows but very few coils. The tip is usually slightly enlarged although occasionally it is conical. It often lies thrust slightly out of the sac and except for the smaller diameter seems to be but little modified from the Vas deferens. A trimming or border of fine chitinous points, similar to those already described for *T. saginata* by LEUCKART (1886, p. 440) cannot be definitely made out. In one or two cases very delicate markings were discernable, but whether they were points or simply wrinkles could not be determined. The representation in the drawing (Fig. 7 *c*) is somewhat exaggerated. The cirrus is so arranged that in extention the seminal duct is evaginated or turned inside out for a short distance, forming the copulatory organ. To be more explicit, the outer end of the cirrus pouch is reflected back, forming a pit which is continuous with the seminal duct.

When the muscular wall of the sac contracts a regular prolapsus of the duct results and its inner side is forced out to the exterior. This process is well illustrated by beginning at the base of a gloved finger and gradually turning it inside out; although of course in the case of the worm the pressure is distributed over what corresponds to the entire outer surface of the glove finger, thus forcing the tube to double back upon itself and turn inside out. This arrangement is also found in *T. saginata.*

The cirrus pouch (Fig. 7 *c.p*) is somewhat pear-shaped and measures 0.31 mm in length. Its walls are made up largely of muscular, web-like structure in which the fibres run diagonally. On the inside are retractor muscle fibres (Fig. 7 *r.m.c*) which attach the cirrus to the wall, and also, many connective tissues fibres. Numerous deeply staining bodies, which for want of a better name are called free nuclei* (Fig. 7 *f.n*) are present and are distributed throughout the inner network of fibres. In addition to the nuclei, numerous other minute bodies which resemble them very closely are present. Upon close examination the latter are seen to be the cut ends of small muscle fibres. These small bodies (both nuclei and small muscles fibres) are found also along the edge of the pore plug and the border of the vagina (Fig. 7). The seminal vesicle, as described above, also lies inside of the sac.

The genital pore. The genital pore (Fig. 4 *p*) or generative cloaca as it is sometimes called, is very different in structure from that of either *T. saginata* or *solium* (compare Figs. 7, 8). The pore in the majority of cases is distinct but not prominent and measures in the sexually mature proglottids about 0.45 mm in diameter by only 0.05 to 0.08 mm deep. This extreme shallowness is almost sufficient ground in itself to exclude this form from belonging to the above named species; for example, *T. saginata*, according to LEUCKART (1886, p. 440), has a wide pore or a funnel-like cavity, 0.22 mm deep (Fig. 8 *p*). The pore in *T. confusa*, contains a large plug-like projection (Fig. 4 *pp*) at the outer extremity of which the seminal duct opens. This plug is so large in fact that the cloaca is almost obliterated (Fig. 7) being only a slight depression left between the edge of the plug and the lip of the pore, due to the rounding off at the end of the former. It generally extends out even with the edge of the proglottid and sometimes a trifle beyond. As mentioned above, the cirrus opens out about its center and when not protruded there is a slight invagination or pit at the end of the plug. The cirrus when thrust out, is wholly outside the cavity, judging from which, one is inclined to think that copulation between the organs of the same proglottids does not normally occur. The vagina, to be described later, opens back of the plug, between it and the posterior edge of the cloaca.

The various muscles layers of the wall are little if any changed in the region of the pore. No special sphincter muscles for the whole pore is present. The slight pit which forms the cloaca, is dipped into by the cuticula (Fig. 7 *c.u*) which then turns up the side of the plug and finally down into the cirrus opening. Thus the cirrus and Vas deferens are lined by a continuation of the body wall.

The pores are not regularly alternating on the proglottids but in the main, in a given number, they about balance (Fig. 1). They always lie back of the middle of the segment, this arrangement becoming more pronounced as the proglottids increase in length. They increase but slightly in size with the increasing age of the proglottid and in the long ripe segments, in which the central plug has about disappeared, they are more or less shrunken or collapsed internally, still however, possessing a distinct outer lip or ring.

In a few cases, in segments where the uterus is just beginning to branch, the pore is a trifle deeper than in most of the sexually mature ones, measuring about 0.09 mm. It is also deeper in transverse section than longitudinal (compare Figs. 6 and 7), thus showing that there is more of an indenture on the dorsal and ventral sides of the plug than on the anterior and posterior sides. The following measurements will give some idea of the relative size of the pore and plug in sexually mature proglottids. Dorso-ventrally the plug measures 0.2 to 0.4 mm in diameter, the pore, 0.26 to 0.60 mm. Longitudinally the plug measures 0.3 to 0.4 mm, the pore 0.5 to 0.6 mm in diameter.

Female Organs.

Vagina. The pore in addition to receiving the male duct is also the terminus for the female canal, or vagina, which opens directly behind the plug (Fig. 7 *v*). The vagina is seen as a long thread-like structure, more or less sinuous but showing little tendency toward the formation of complicated coils like those so characteristic of the Vas deferens (Fig. 3 *v*). It extends into the proglottid some distance parallel to the last mentioned organ, and then makes a quarter circle to the rear, where finally, after undergoing some modifications of structure, it terminates in the shell-gland. The terminal end which opens into the pore is also considerably modified, being much enlarged in diameter. At this point, it springs rather abruptly from its normal narrow state (about 24.5 μ) to a breadth of 0.04 to 0.06 mm, and then gradually tapers to the outer opening. The narrowing of the canal again, which varies considerably in different proglottids is due probably to the state of contraction of the pore itself. The enlargement begins about 0.25 to 0.60 mm back from the outer opening and has scattered along its walls, a great number of free nuclei (Fig. 7 *f.n*) similar to those of the cirrus pouch. These are also present along the entire length of the vagina.

A short distance from the exterior, a well developed sphincter muscle (Fig. 7 *sp.m*) is found, which in section measures from 0.02 to 0.035 mm across the cut end. It extends as a band or ring, completely around the vagina and is present in all proglottids. It serves, probably, to hold the cirrus firmly during copulation. In the sexually mature proglottids, the lumen of the vagina is usually considerably narrower at the point where the sphincter surrounds it.

Since the presence of such a muscle in either of the other human

Taeniae, has never been mentioned, to my knowledge, I considered this structure at first, as characteristic only of *T. confusa*, but upon examining my specimens of *T. saginata* I found a prominent sphincter muscle present in relatively the same position (Fig. 8 *sp.m*). It differs from that of *T. confusa* in being broader and more band-like, and like the other muscles of *T. saginata* is coarser and more powerful. As in *T. confusa* the vagina is narrower in its immediate vicinity.

Such a muscle as the above, has been found in two of the other than human Taeniae but its presence in *T. saginata*, I think, has never before been recognized. In preparations from a very large specimen of a peculiar human *Taenia*, which I have in my possession, a vaginal sphincter muscle is very distinctly seen; but whether this tapeworm is merely a highly modified form of *T. saginata* or a different species, I am as yet unable to say.

The greater part of the vagina, the canal connecting the modified ends is, in appearance, much like that of *T. saginata* with the exception of the cilia, if, indeed, cilia are present in the new form. As a matter of course, on general principles, one expects to find the vagina of the Taeniae ciliated, but that such is the case in this form, I am by no means assured. Certain structures are present which at first sight appear to be cilia, but upon very close examination their interpretation becomes decidedly doubtful. They do not have the characteristic fringe-like appearance that cilia usually possess, and instead of pointing toward the receptaculum as do cilia in the ordinary forms these point outward toward to pore (Figs. 7—9 *v*). In fact, they have more the aspect of numerous tiny folds in the wall of the vagina than of cilia and I am rather inclined to think that they are such. Although I have carefully examined all my preparations, I find it impossible, without further evidence, to affirm positively either that the structures are, or are not, cilia.

Like the Vas deferens the vagina is lined by a continuation of the cuticula, which, however, is considerably thicker than in the former organ. The tube, as above indicated, measures about 24.5 μ in diameter, the lumen about 19.5 μ. More or less of a special case or sheath-like structure (Fig. 7) surrounds it, inside of which is a sort of connective tissue network or system of fibres very similar to those surrounding the cirrus, and likewise having free nuclei plentifully distributed throughout. As the proglottids grow older, the deposits of pigment, which are not very plentiful in the younger proglottids, increase, rendering the vagina more and more prominent. It is plainly visible in the very terminal proglottids.

Receptaculum seminis. A short distance before the vagina enters the shell gland, there is a noticeable increase in the diameter and the walls are slightly modified in structure. This enlarged portion is the seminal receptacle (Fig. 9 *r. s*) and is for storing the spermatozoa which pass up through the vagina from the genital pore. In this species it is almost circular in frontal section (0.135 mm in diameter) and of a lenticular shape in transverse section (0.078 mm in diameter).

Before leaving this point, there is a peculiar structure connected with the receptaculum which should be mentioned. Leuckart in his description of *T. saginata* (1886, p. 444) pictures a slightly modified structure just anterior to the receptaculum, but beyond mentioning the fact that the latter is rather peculiarly connected with the vagina anteriorly, gives no description or explanation of the same. In my own preparations of *T. saginata*, however, I find no such modification. The structure referred to in *T. confusa* lies immediately in front of the seminal receptacle (Fig. 9 *x*). Shortly before reaching the receptacle, the vagina becomes slightly enlarged and then abruptly narrowed or rather, the narrow part seems to be of almost entirely different structure, and projects forward into the slight swelling in the vagina just mentioned. The walls of the new structure are thicker and the lumen much narrower than those of the vagina proper. The interior is apparently of the same structure as that of the latter. The walls are surrounded by what appear to be great numbers of small sphincter muscles (Fig. 9 *sp. m*), which are very distinctly seen in the longitudinal sections. The free nuclei mentioned several times heretofore, are very numerous in this vicinity. This constricted part of the vagina continues back a distance of about 0.11 mm to the easily recognized receptaculum, which is always filled with spermatozoa in the sexually mature proglottids.

The continuation of the vagina, if we regard the seminal receptacle as an enlargement of it, is a wide thin walled canal, comparable in structure to the "Befruchtungs"-canal of Leuckart (1886, p. 442) in *T. saginata*. This extends back to the shell gland (Fig. 10, *s. g*), an oval body located about midway between the lobes of the ovary. Before it reaches the shell gland, however, it receives a short canal (0.1 mm long) from the cross band connectiug the two lobes of the ovary (Fig. 10 *z*).

Shell gland. The shell gland, as above mentioned, is an oval body measuring about 0.25 mm long by 0.2 mm wide, and is the common center for all the canals of this region. The peculiar radiate appearance it has in section is due to the granular cells which are packed together, side by side, with their narrow ends or ducts pointing in toward the center of the organ. Extending vertically through its middle is a narrow duct which slightly enlarges near the center (Fig. 10 *s. g*). This enlargement measures 0.02 mm wide by 0.068 mm long. The duct is a direct continuation of the above mentioned wide thin-walled modification of the vagina connecting the receptaculum and shell gland. From the anterior ventral side of the shell gland, after being joined by the duct from the vitellaria (Fig. 10 *vl*) which comes in from the posterior side, it bends directly upward, passes through the center of the gland and after reaching the dorsal edge, doubles back very slightly upon itself and extends forward as the canal which bears the eggs to the uterus (Fig. 10 *y*). Unlike the case in *T. saginata* or *T. solium*, this canal does not enter the posterior end of the uterus, but extends along above it for some distance, gradually declining until it finally enters the dorsal side of the same.

The ovary. This is a paired organ of about the same general structure as that of *T. saginata* or *T. solium*, but differing very much from either in form (Fig. 3 *o*). The wing-like expansions or lobes are greatly elongated, kidney-shaped and larger than the corresponding structures of the other Taeniae. They include between them the area containing the receptaculum seminis, the shell gland and the end of uterus. As stated, they are much longer than broad, the larger one, which lies always on the opposite side from the vagina measuring in general about 1.4 mm long by 0.56 mm broad. This makes it about two and one half times as long as broad. The one lying on the same side as the pore is generally about 0.1 mm shorter than its mate, measuring only about 1.3 mm in length.

The lobes are connected by a broad (0.2 mm) transverse band (Figs. 9 and 10 *b*) which springs from about their median inner edges and passes across the intermediate field just under the posterior edge of the receptaculum. This arrangement of the cross canal is the same as that of *T. saginata* or *T. solium* according to the statements of Sommer (1874, p. 26) for the latter forms, but different from what is given for *T. saginata* in the translation of Leuckart (1886, p. 444) where is said that the cross canal passes over instead of under the receptaculum. This statement attributed to Leuckart is probably simply a mistake in translation, inasmuch as in the German editions he speaks of the canal in question as passing under (ventral to) the vagina. At about the middle of the band, a small duct is given off as already described, which runs only a short distance till it connects with the modified parts of the vagina extending from the seminal receptacle to the shell gland (Fig. 10 *s*). It is very slightly coiled and generally enters the vagina somewhat from the side.

The vitellarium. The vitellarium or yolk gland (Figs. 3—10 *vt*) is a triangular, unpaired organ, extending out laterally about even with the edge of the ovarian lobes, and presenting no new or unusual modifications of structure. It shows a more or less scalloped edge posteriorly, but anteriorly wedges in between the ovarian lobes and tapers off toward the shell gland. Its duct finally enters the gland at the ventral posterior edge (Fig. 10) and immediately joins the vertical canal through the same. As for the contents of the vitellaria they may be easily recognised from those of the ovary, because of their much more finely granular nature.

The uterus. The connection of the uterus with the shell gland has been explained already, but as yet little has been said of the structure of the uterus itself. It has, however, an exceptional structure, which defeats beyond all doubts any attempt to classify its possessor as one of the common forms of *Taenia*.

In the proglottids ready for fertilization the uterus is a median unbranched tube ending blindly near the anterior end, and extending back posteriorly between the lobes of the ovary to the shell gland (Fig. 3 *u*). The very great difference in structure in the neighborhood of the shell gland, between it and *T. saginata* or *T. solium*, has already been pointed out. The latter have uteri which are very similar in the unbranched state. They extend back and end near the anterior edge of the lobes of the ovary, each connecting with the shell gland by means of a slender canal which opens into its posterior end. In the case of *T. confusa*, the canal (Fig. 10 *y*) opens into the dorsal side of the uterus, and the latter extends back until it lies directly against the shell gland (Fig. 10 *u*). This arrangement may be seen in the proglottids from the time that they become sexually mature until the disappearance of the shell gland. In its primitive unmodified form (Fig. 3 *u*) the uterus is about 0.15 mm in diameter.

As the uterus fills with eggs it begins to bud out thickset and stubby side-branches which usually persist in the ripe segments as heavy, arborescent members (Fig. 2). In a few of the terminal proglottids from which a part of the eggs have escaped the branches often become thin near the point of attachment to the main stem.

The branching begins first at the anterior end of the uterus and gradually extends backward, the eggs seeming to pass down the tube and mass at the anterior end. As the branches increase in number and size, the proglottids lengthen and the generative organs, for the most part, gradually disappear. Finally, the uterus with its offshoots comes to occupy the entire field within the limits of the excretory canals (Fig. 2).

In the ripe proglottids, the uterus consists of a main stem with from 14 to 18 lateral branches which are generally very irregularly disposed throughout the parenchyma, rarely running out perpendicularly to the main stem, as is the common method in *T. saginata.* Usually they extend only a very short distance before they split up into two and sometime three branches which again often divide in a similar way. They seldom extend in a straight line but have a more or less pronounced sinuous outline. More resemblance to the proglottids of *T. solium* is shown, than to those of *T. saginata*, but the uterus extends forward to the anterior end of the proglottid, and the number of branches is greater than in *T. solium.* The ends of the branches are usually swollen into large, irregular, club-shaped masses, which border on the excretory canals. At the anterior end, there are usually from three to seven peculiar tassle- or vase-shaped branches, which extend forward to the very edge of the proglottid. They are so arranged that when two proglottids are separated, the end of the uterus is torn open and the eggs set free. According to LEUCKART (1886, p. 424), the eggs are set free at the anterior border of the proglottid in *T. saginata* also. At the posterior end as in *T. saginata* the uterus falls short of the edge of the proglottid by from 2 to 3 mm.

The branches at the anterior and posterior ends, especially the latter, are longer than the others and lie more obliquely to the long axis of the segment. The posterior ones are also usually more highly branched and fuller on the outer side, in consequence of which, they have the appearance of bending or drooping into a sort of plume-like shape. This is especially marked in the detached ripe proglottids. In these also, the central stem is sometimes very thin and the branches, as already mentioned, of very small diameter at their points of attachment. There seems to be no omission of branches on the side occupied by the pore, although the ones lying in its vicinity are ordinarily not so heavy as the corresponding ones on the opposite side.

Sexual development. The first definite appearance of the sexual organs occurs in the small anterior proglottids measuring 0.8 mm long by 0.6 mm wide.

The first indication seen is a transverse streak about the middle of the proglottid, extending from near the median longitudinal line to within a short distance of the edge, and bearing at the outer end a club-like swelling. The streak is the "Anlage" of the vagina and vas deferens, and the enlarged end will ultimately develop into the sexual pore. The development goes on very rapidly, for in proglottids a very short distance further back, the streak has grown to form a quarter circle, the inner end of which extends toward the posterior end of the proglottid. Soon, in proglottids measuring 1.2 mm long by 0.84 mm wide, a sort of a Y is formed with the short arm extending forward. In this stage the largest diameter of the developing organs is at a point near the edge where the future pore will develope. Here also, the first indications of the differentiation into the vagina and vas deferens are seen, the intermediate substance becoming gradually thinned out. In proglottids measuring 1.6 mm long by 1.9 mm wide, an almost complete separation of vas deferens and vagina has taken place. They still, however, remain in contact in the deeply staining, club-like portion which is the Anlage of the pore. In this stage the uterus has extended forward as far as it ever does before branching, but has not yet attained the width it reaches in the sexually mature proglottids. It bends somewhat out of its course to pass around the end of the vas deferens, and then back at a sharp angle resuming its former course until it finally connects posteriorly with the ending of the vagina, which shows two enlargements in about the region where the receptaculum and shell gland will appear. By this time the joined end of the vagina and vas deferens have reached the lateral edge of the proglottids.

In proglottids measuring 1.7 mm long by 1.8 mm wide the ovary first makes its appearance, as a granular bean-shaped area. The testes have also put in appearance and are very little if any older than the ovary. In these proglottids the pore has established its connection with the exterior and the cirrus pouch is formed. The vas deferens has somewhat the appearance of a thread within an outer tube or case. The sheath around the vagina is also discernible. At this period, as in all the later, the uterus extends back entirely to the shell gland, and the dorsal and ventral sides of the proglottids can be readily distinguished.

As for the sexually mature and ripe proglottids, since they have been rather fully treated already, little more need be said about them in this connection. The branchings of the uterus begin as bud like projections from its sides and as these increase the sexual organs gradually disappear; the testes first, followed by the ovary and the vitellaria. As has been stated already, the vas deferens and the vagina, more especially the later, persist for a much longer time (Fig. 2 *v.d*, *v*). The shell gland usually remains for a considerable time, leaving here as in *T. saginata*, upon its disappearance a vacant area free from branches.

Eggs. The eggs in the ripe proglottids are oval in form and are made up of several different parts (Fig. 11). First on the outside, is a thin transparent membrane, and next a layer of little rods standing side by side. Just inside the rods comes a thin layer or space, the exact nature of which was not determined. The elongated inner portion is of about the same general outline as the outer covering of the egg, and is of different appearance in different eggs. In some there is a sort of dark cap-like structure at one end, in others it is present at both ends, and in others yet, the dark area extends along the side and one end, or often both ends. Usually the entire center is dark. In no case can the pyriform apparatus, or tail-like processes spoken of by LEUCKART (1886, p. 449) in connecting with the eggs of *T. saginata* be found. It is not unreasonable to, suppose however, that since they are very delicate, they may have been present, but were destroyed through the poor preservation of the material. The eggs measure in general about 39 μ long by 30 μ wide.

The development of the same has not as yet been worked out. For comparison with eggs of *T. solium* and *T. saginata* see the table below.

It has been suggested that possibly this species is the same as that, from a North American Indian, which WEINLAND described in 1858. If, however, the descriptions of the two forms are compared, it will be found that they agree in scarcely any particular. The head, neck and whole anterior half of his specimen were wanting. The following summary is taken directly from WEINLAND's description of the form in his possession (1858, p. 44).

1) Joints very thin, nearly transparent, equally narrow, transverse diameter about 4 mm, longitudinal about 12 mm.

2) Genital openings very small and without external lips.

3) No pigment in either vagina or spermatic duct.

4) Uterus more regular than either *T. solium* or *T. medicanellata*, yet more resembling the latter.

5) Branches of uterus, about 30 in number start from the main stem, either at right angles or at angles of about 45 degrees.

6) Branches of uterus always quite parallel, and generally straight; but whenever bent, all makes the same angle.

7) Branches of uterus never arborescently divided, nor furcated at the ends, except the foremost and hindermost in each joint.

8) Eggs 0.033 mm long by 0.030 mm broad.

Comparing the above summary with the following which embraces corresponding anatomical points in the structure of *T. confusa*, but little similarity between the two forms is seen to exist.

1) Joints thin, not transparent, equally narrow, transverse diameter 3.5 to 5 mm, longitudinal 27 to 35 mm.

2) Genital openings not exceptionally small and with very distinct external lips.

3) Deposits of pigment, plentiful in both vagina and spermatic duct, the former being especially prominent in most of the ripe proglottids.

4) Uterus less regular than that of either *T. solium* or *T. mediocanellata*, yet more resembling the former.

5) Branches of the uterus from 14 to 18 in number, which start from the main stem very irregularly as regards angles.

6) Branches of uterus rarely parallel or straight, whenever bent, they exhibit no regularity in the shape of the angles.

7) Branches of uterus often arborescently divided, and often furcated at the ends throughout the entire proglottid.

8) Eggs 0.039 mm long by 0.030 mm wide.

The following table is a summary of the principle points of difference between *T. confusa* and the two common forms of *Taenia* found in the human alimentary canal. The measurements for *T. saginata* and *T. solium* are quoted for the most part from LEUCKART (1888):

	T. saginata	*T. solium*	*T. confusa*
Total length of the worm	4—8 m	2—3.5 m	5—8 m
Total no. of proglottids	1300	850	750—800
Size of terminal proglottid	12—19 mm long, 5—6,5 mm wide	10—12 mm long by 5 mm wide	27—35 mm long by 3.5—5 mm wide
Change of form in the terminal proglottid at anterior end	very considerably	slight	very slight
Greatest breadth of chain	12—14 mm	7—8 mm	8—10 mm
Length of proglottids exceeds breadth	only in last 100	in last half	in nearly the entire worm
Proglottids 35 cm behind the head measure	1.5—2.6 mm long, 5—10 mm wide	0.8 mm long, 1.3 mm wide	1—2.5 mm long, 0.8—3 mm wide
Sexually mature proglottids measure	4—6 mm long, 8—10 mm wide	2.5—3 mm long 4.5—5 mm wide	4—4.5 mm long, 3.5—4.5 mm wide
Sexually mature proglottids show	disproportionate increase in breadth	o.	an increase in length with slight increase in breadth
Diameter of head	1.5—2 mm	1 mm	unusually small
Necks	unsegmented	unsegmented	segmented
Thickness of cuticula	* 0.0105 mm	—	0,0045 mm
General musculature	powerful	delicate	delicate
Longitudinal muscle fibres	not continuous between proglottids	do.	continue from one proglottid to the next
Calcareous bodies	plentiful, measure up to 0.018 mm	sparse, measure up to 0.12 mm	sparse, measure up to 0.011 mm
Longitudinal nerve strands	dorsal to genital ducts	do.	divide, passing both dorsal and ventral to genital ducts
Seminal vesicle	absent	absent	present
Diameter of testes	0.15 mm	0.12—0.15 mm	0.089—0.096 mm
Diameter of vas deferens	0.025 mm (0.040)	†	0.045 mm
Length of cirrus pouch	0.4—0.5 mm	†	0.31 mm
Depth of generative cloaca	0.22 mm	†	0.05—0.08 mm
Width of pore	1 mm	†	0.45—0.6 mm
Receptaculum seminis	0.1 mm long by 0.07 mm thick	†	0.135 mm long by 0.078 mm thick
Vagina	ciliated	† ciliated	doubtful, at least modified
Posterior part of vagina	little if any modified anterior to recptaculum	—	greatly modified anterior to receptaculum and provided with sphincter muscles
Ovary	lobes round	part of smaller one cut off by vagina. Lobes transversely oval	lobes kidney shaped
Connection of uterus with shell gland	thin duct opens into end of uterus	duct longer and connects same as in *T. saginata*	duct opens into the side of the uterus, posterior end of uterus lies against shell gland

	T. saginata	*T. solium*	*T. confusa*
Uterus in terminal proglottids	20—30 branches	7- 10 branches	14 – 18 branches
Branches of uterus	branch at right angles and are slender	branch at right angles and are thick	often do not branch at right angles and are generally thick turned and crooked
Branches of uterus absent on pore side	usually 2	—	usually the same number on both sides
Size of eggs	slightly oval, 0.03 mm in diameter	almost round, 0.03 mm in diameter	oval, 0 039 mm long by 0.03 mm wide
Pyriform process	present	present in young proglottids	absent apparently
Eggs	escape at anterior border of proglottids	do not escape at anterior border	escape at anterior border
Color of eggs	rust-brown	usually brown	whitish or yellowish
Sexual development seen first in proglottids	0.8 mm long by 2.5 mm wide	1.5 mm long by 2.2 mm wide	0 8 mm long by 0.6 mm wide
On the first Anlage the club-like end of the newly forming vagina and vas deferens lies	inward, toward uterus	inward	outward

* According to my own measurements.

† In speaking of the reproductive organs of *T. solium* LEUCKART (1886, p. 526) says that they are smaller in dimensions than those of *T. saginata.*

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Explanation of the Plate.

b	band connecting two lobes of the ovary
c	cirrus
c.p	cirrus pouch
cu	cuticula
f.n	connective tissue nuclei
l.e.c	longitudinal excretory canal
l.n.s	longitudinal nerve strand
l.m	longitudinal muscle fibres
o	ovary
p	pore
p.p	pore plug
r.m.c	retractor muscle of cirrus
r.s	receptaculum seminis
s.g	shell gland
s.m	sagittal muscle fibres
sp.m	sphincter muscle
t	testes
t.e.c	transverse excretory canal
t.m	transverse muscle fibres
u	uterus
v	vagina
v.d	vas deferens
v.s	vesicula seminalis
vt	vitellaria
x	thick walled tube connecting the vagina with the receptaculum
y	canal through which the eggs pass from the shell gland to the uterus
z	canal which carries the ovarian eggs toward the shell gland

Fig. 1. *Taenia confusa* WARD (nat. size). *a* Anterior end a short distance back of head, *c* sexually mature proglottids, *f* terminal proglottids.

Fig. 2. Ripe proglottid (× 6).

Fig. 3. Sexually mature proglottids (× 20) showing the reproductive organs.

Fig. 4. Transverse section of sexually mature proglottid (× 40).

Fig. 5. Sagittal section through the connecting isthmus of two proglottids (× 50).

Fig. 6 Transverse section through the pore region of a sexually mature proglottid (× 50).

Fig. 7. Frontal section through the pore region of a sexually mature proglottid (× 160).

Fig. 8. Frontal section through the pore region of *T. sagi* (× 160).

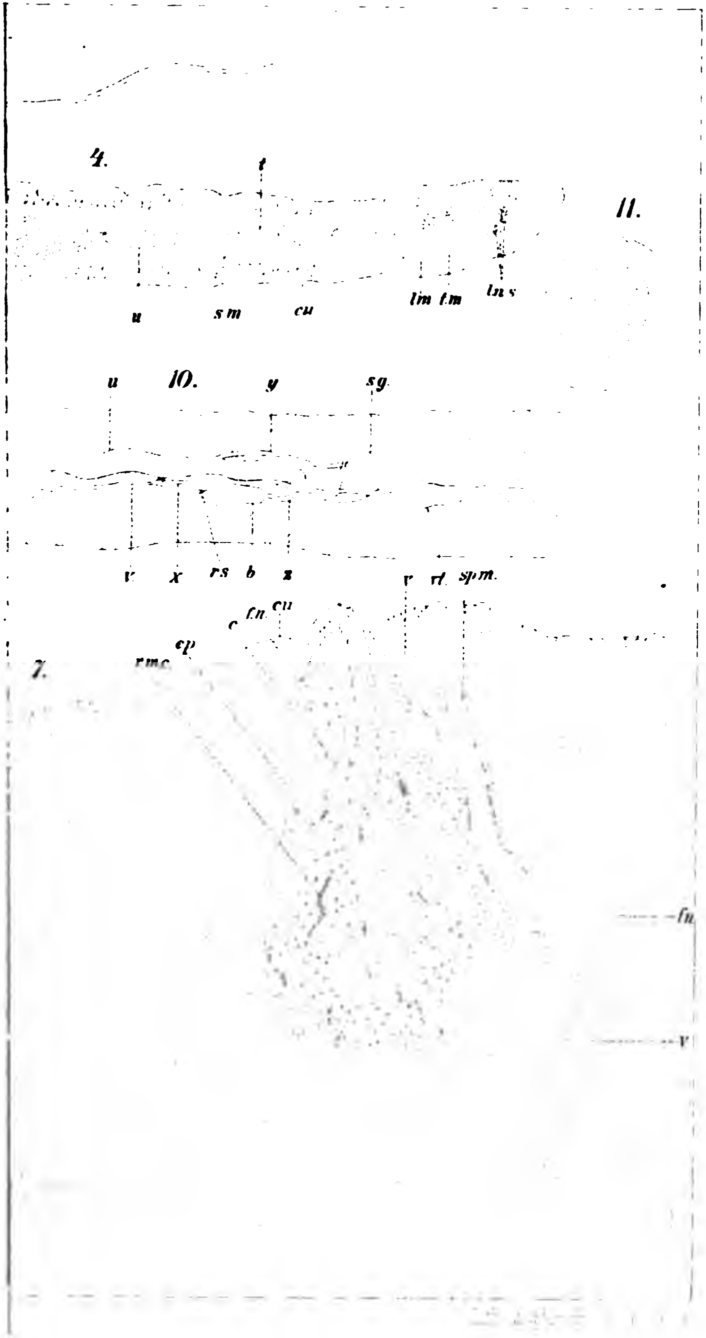
Fig. 9. Frontal section showing posterior modifications of vagina (× 160).

Fig. 10. A diagram built up by means of the camera lucida fr consecutive sagittal sections showing the relative size and position the female sexual organs (× 50).

Fig. 11. Egg from ripe proglottid (× 660).

Errata.

p. 2, l. 11, after "it", add comma; after "many" add "respects".
„ 3, l. 4, after "other" add comma; l. 5 after "proglottids" add comma.
„ „ l. 9—10 omit "being arranged".
„ 4, l. 21 instead of "later" read "latter".
„ 5, l. 15 after "with" insert "that".
„ „ l. 25 after "correspondingly" add comma; after "4.5 μ" ad semicolon.
„ „ l. 6 from bottom after "fibres" add comma.
„ „ bottom line should read "the center. One end" etc.
„ 6, l. 9 from bottom after "moreover" add comma.
„ „ l. 5 „ „ „ "saginata" remove comma.
„ 7, l. 9 omit "the" at the end of line.
„ „ l. 8 from bottom after "part" add comma.
„ 8, l. 9 for "larger" read "large".
„ „ l. 15 for "cross" read „gross".
„ „ l. 24 at end of line read semicolon instead of comma.
„ 9, l. 3 after "forms" insert comma.
„ „ l. 17 for "Fig." read "Figs."
„ 10 middle, do not make a paragraph between "duct" and "When".
„ „ l. 6—7 from bottom instead of text read "which consists principally of connective tissue nuclei (Fig. 7 *f. n.*)".
„ 11, l. 26 for "muscles" read "muscle".
„ 12, l. 11 from bottom for "free nuclei" read "connective tissue nuclei".
„ 14, l. 13 „ „ „ „ „ „ „ „ „
„ 15, l. 12 for ("Fig. 10 *v.l*)" read "(Fig. 10 *v.t*)".
„ „ l. 2 from bottom after "where" insert "it".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FACULTÉ DE MÉDECINE DE PARIS

Année 1901 **THÈSE** N°

POUR LE

DOCTORAT EN MÉDECINE

Présentée et soutenue le Jeudi 28 Février 1901, à 1 heure

PAR

J. GUYOT

LICENCIÉ ÈS-SCIENCES PHYSIQUES ET NATURELLES
CHEF DES TRAVAUX PRATIQUES DE ZOOLOGIE A L'UNIVERSITÉ DE RENNES.
Né à Noyalo (Morbihan), le 16 Avril 1866.

CONTRIBUTION A L'ÉTUDE DES LARVES DE GASTROPHILES (ŒSTRIDES) PARASITES DE L'ESTOMAC DU CHEVAL

Président : M. R. Blanchard, professeur.
Juges : MM. Debove, professeur
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Par délibération en date du 9 décembre 1798, l'Ecole a arrêté que les opinions émises dans les dissertations qui lui seront présentées, doivent être considérées comme propres à leurs auteurs et qu'elle n'entend leur donner aucune approbation ni improbation.

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A M. le Professeur R. BLANCHARD

Membre de l'Académie de Médecine,
Professeur à la Faculté de Médecine,
Chevalier de la Légion d'honneur,
Secrétaire général honoraire de la Société Zoologique de France.

A mon premier Maître

M. le Professeur L. JOUBIN

Professeur à l'Université de Rennes.

Hommage respectueux et reconnaissant.

INTRODUCTION

Parmi les parasites qui s'attaquent à l'Homme ou aux animaux, il est une catégorie d'Insectes, les Œstrides, dont les larves présentent cette curieuse particularité de se développer dans le tube digestif des Solipèdes. Alors que comme Insectes ailés ils sont libres et ne semblent vivre que juste le temps nécessaire à la reproduction, à l'état de larves au contraire, ils ont une existence parasitaire très longue, pendant laquelle ils demeurent constamment fixés aux parois digestives de l'hôte aux dépens duquel ils se nourrissent.

Malgré les nombreux travaux qui ont été publiés au sujet de ces singuliers animaux, les premières phases du développement des larves étaient très peu connues jusqu'à présent et les lésions qu'elles occasionnent n'avaient été l'objet d'aucune étude spéciale. Je me propose dans ce travail d'exposer le résultat des recherches que j'ai faites sur ces deux questions ; j'y ajouterai les quelques observations que j'ai recueillies relativement aux œufs et aux larves ayant atteint leur complet développement.

Avant d'entrer en matière, je suis heureux d'adresser l'expression de ma profonde gratitude à M. R. Blanchard, professeur de Parasitologie à la Faculté de Médecine de Paris, qui a été l'inspirateur de ce travail et qui, en plus, a bien voulu se charger de faire reproduire les dessins et photographies qu'il renferme.

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HISTORIQUE

Depuis les temps les plus reculés on connaît les Insectes diptères qui s'attaquent aux quadrupèdes domestiques. Les grecs désignaient sous le nom d'οἶστρος et les latins sous celui d'*Asilus* un animal qui causait un indicible effroi aux troupeaux de Bœufs; mais les auteurs ne sont pas d'accord pour savoir s'il s'agissait bien réellement d'une Mouche de la famille des Œstrides. D'après Joly (**16**), il est probable que si les anciens ont connu les vrais Œstrides, à l'état parfait, ils les ont confondus avec les Taons et surtout avec le *Tabanus bovinus* L., dont la présence cause une grande terreur aux troupeaux de Bœufs.

Quant aux Œstrides à l'état de larves, il est certain qu'Aristote (**1**) connaissait celle qui vit dans le gosier des Cerfs, puisqu'il la décrit dans son Histoire des animaux.

Les vétérinaires grecs Theomnestus, Absyrtus ont vu les larves qui se fixent à l'anus des Chevaux ; ils les prenaient pour des Vers rongeurs qu'ils désignaient sous le nom de Térédines (τερηδινες) et vont même jusqu'à conseiller, pour les détruire, de les arracher de l'anus avec les doigts et de les tuer en les couvrant de cendres chaudes et de sel pulvérisé.

Pour avoir des faits positifs, il faut arriver à la fin du 17^e siècle. Malpighi (**22**), le premier, en 1697, décrit une larve provenant de l'estomac d'un Ane. D'après Joly (**16**), elle appartiendrait à l'espèce *Gastrophilus intestinalis* (Degeer) Leach, tandis que pour Brauer (**6**), ce serait le *Gastrophilus flavipes* Oliv.

Gaspari émit cette opinion erronée que le Gastrophile hémorrhoïdal déposerait ses œufs dans le rectum du Cheval, au moment de la défécation, et que, de là, les jeunes larves remonteraient jusqu'à l'estomac pour revenir, par la même voie, au rectum où elles finiraient d'acquérir leur maturité.

Vallisnieri (**43**), médecin de Padoue et neveu du célèbre Malpighi, en dehors de ses autres travaux sur les Œstrides, ajouta quelques détails à l'histoire du Gastrophile du Cheval ; mais il contribua puissamment à propager l'erreur dans laquelle était tombé son compatriote Gaspari.

Réaumur (**34**) adopta la même erreur ; malgré cela, ses travaux étendus sur les larves de l'Œstre du Bœuf, du Mouton, du Cerf et du Cheval sont demeurés classiques.

Ce fut Linné (**21**) qui donna à la famille le nom générique de *Œstrus,* mais il attribua à l'Œstre du Cheval la larve de l'Hypoderme du Bœuf ; les caractères de son *Œstrus bovis* s'appliquent au *Gastrophilus intestinalis* (Degeer) Leach et non pas à l'*Hypoderma bovis* Degeer.

Fabricius (**12**) commit la même faute et ne la corrigea que beaucoup plus tard, en 1805.

Le baron Degeer (**10**) distingua, le premier, les deux espèces et fit de l'*Œstrus bovis* de Linné, c'est-à-dire du *Gastrophilus equi* (Leach) Clark une description exacte sous le nom d'*Œstrus intestinalis.*

C'est seulement en 1797, avec Bracy Clark (**4**), célèbre vétérinaire anglais, que commence véritablement l'histoire naturelle des Œstrides. Cet auteur divisa les Œstrides en trois groupes basés sur les lieux où les larves séjournent et se développent : 1° les *Gastricoles,* chylivores; les *Cuticoles,* purivores ; 3° les *Cavicoles,* lymphivores. Il s'occupa spécialement des Œstres des Chevaux, décrivit leurs mœurs, fit connaître les remarquables migrations de leurs larves et corrigea beaucoup d'erreurs anciennes.

Il faut ensuite arriver à l'année 1863 pour avoir, avec Numan (**28**), des détails intéressants sur les larves d'Œstrides qui séjournent dans l'estomac des Solipèdes. Il est le premier à signaler les mues chez les Gastrophiles en découvrant dans l'estomac d'un cheval, mélangées aux larves rouges de son Œstre hémorrhoïdal, de petites larves, rouges également, encore recouvertes de leur ancienne peau. Mais, n'en ayant pas fait une étude suffisamment approfondie, il ne put savoir à quelle espèce elles appartenaient.

Jusque-là on ne s'était guère occupé que de la partie descriptive des Œstrides : la première étude importante sur l'anatomie de ces animaux fut publiée, à peu près en même temps, par Schroeder van der Kolk (**41**) et par Joly (**16**). Le premier s'occupa spécialement de la larve de *Gastrophilus intestinalis* (Degeer) Leach qu'il appelle *Gastrus equi ;* le second étendit, en outre, ses recherches aux larves de *Gastrophilus hæmorrhoïdalis* (Leach) Clark, aux Insectes parfaits de ces deux formes ainsi qu'aux Insectes parfaits et aux larves de plusieurs autres espèces d'Œstrides. Joly, le premier, décrivit et figura la jeune larve de *Gastrophilus intestinalis* (Degeer) Leach au moment de son éclosion, mais d'une façon incomplète et en partie inexacte.

Deux autres auteurs, Meinert (**26**) et Scheiber (**37**), ont également traité de l'anatomie du Gastrophile ordinaire du Cheval. C'est seulement dans le travail de ce dernier qu'il est question d'une note très courte de Wedl (**44**) sur la nature des blessures provoquées par la larve de *Gastrophilus intestinalis* (Degeer) Leach sur la muqueuse gastrique du Cheval.

En 1863, Brauer (**6**) publia son importante Monographie des Œstrides dans laquelle il traite de toutes les espèces connues jusqu'alors, mais sans parler aucunement de leur structure anatomique. C'est le dernier ouvrage important, et aussi le plus complet, qui ait paru sur la question.

Depuis cette époque jusqu'à nos jours, malgré les travaux de Gayot (**15**), Semmer (**40**), Brown (**8**), Laboulbène (**18**), Poujade (**30**), Joly (**17**), Enderlein (**11**), Prenant (**31** et **32**), etc., le cercle de nos connaissances s'est très peu élargi. Je mentionnerai seulement le travail de Perroncito (**29**) au sujet d'un traitement par le sulfure de carbone qui, paraît-il, serait efficace pour déloger les larves de Gastrophiles de l'estomac du Cheval. Mais je n'ai trouvé aucun mémoire relatif aux premières phases du développement larvaire, ni aux lésions déterminées par les larves des Œstrides gastricoles. Dans la dernière édition de son traité de Zoologie médicale et agricole, Railliet (**33**) mentionne toutefois, d'après Boas, qu'il y aurait quatre stades dans le cours du développement de la larve de *Gastrophilus intestinalis* (Degeer) Leach. N'ayant pu, malgré mes recherches, me procurer le travail de Boas, j'ignore de quelle importance il peut être ; mais si l'on songe que Raillet continue à décrire la larve au premier stade, d'après Joly, et qu'il considère toujours comme peu connues les autres phases, sauf la dernière, je suis tenté d'admettre que les choses en sont restées sensiblement au point où les avaient laissées Joly et Numan. C'est pourquoi je n'hésite pas à publier le résultat de mes travaux sur ces deux questions.

GÉNÉRALITÉS

Les larves que l'on rencontre dans l'estomac du Cheval font partie de la famille des Œstrides, de l'ordre des Diptères, famille des plus remarquables par son organisation et par ses mœurs. A l'état parfait, les Insectes qui la composent ont le port de la Mouche domestique et le corps velu, semblable à celui d'un Bourdon. La tête, assez volumineuse, hémisphérique, est pourvue d'une paire d'yeux à facettes et de trois ocelles placés entre ces derniers. Les antennes, très courtes, possèdent trois articles, le dernier portant un style nu ou plumeux. La trompe, parfois bien développée, mais en général très petite, est accompagnée de palpes rudimentaires ou nuls. L'abdomen, mousse chez les mâles, est souvent prolongé en arrière par un oviscapte chez les femelles.

A cause de leur bouche très petite, ils ne paraissent vivre que le temps nécessaire à l'accouplement et à la ponte, sans prendre de nourriture.

Les femelles déposent leurs œufs sur le corps des Mammifères et donnent naissance à des larves qui sont des parasites nécessaires de ces animaux.

Les larves se transforment en pupes ou nymphes qui, à leur tour, deviennent des Insectes parfaits. Brauer (7) divise les Œstrides en deux sous-familles : les *Œstrinæ* et les *Cuterebrinæ*. Les *Œstrinæ* sont à leur tour divisés, d'après leur habitat, en trois sections : 1° les *Gastricoles*, qui vivent dans le tube digestif ; 2° les *Cavicoles*, qui se développent dans les cavités de la face ; 3° les *Cuticoles*, qui séjournent sous la peau.

C'est dans le groupe des Gastricoles que se place le genre *Gastrophilus*, lequel comprend huit espèces dont trois seulement nous occuperont, parce que ce sont les seules que l'on rencontre en France : *G. intestinalis* (Degeer) Leach, *G. hæmorrhoïdalis* (Leach) Linné, *G. nasalis* Linné.

Afin de rendre la lecture de ce travail plus facile, je vais résumer brièvement leur histoire.

GASTROPHILUS INTESTINALIS (Degeer) Leach.

Synonymie : *Œstrus bovis* Linné, 1761; *Œstrus intestinalis* Degeer, 1776; *Œstrus equi* Clark, 1797; *Gastrophilus equi* Leach, 1817; *Gastrus equi* Meigen, 1824; *Œstrus gastricus major* Schwab, 1840.

Alors que le genre *Gastrophilus* a été créé par Leach en 1817 seulement, Clark (4) avait déjà institué l'espèce *equi* dès 1797 pour désigner l'espèce la plus commune des Gastrophiles du Cheval. Depuis lors presque tout le monde se sert de cette appellation. Cependant, si l'on parcourt l'historique de l'espèce, on trouve, d'après Brauer (6), qu'en 1776, c'est-à-dire longtemps avant Clark, Degeer (10) l'avait déjà décrite sous le nom d'*Œstrus intestinalis*. Le mot *intestinalis* doit donc prévaloir sur celui de *equi*, puisqu'il est le premier en date et qu'il n'a été appliqué à aucune autre espèce du même genre.

Clark rejeta ce nom sous prétexte que la larve vit dans l'estomac et non dans les intestins. Brauer, tout en conservant l'expression de Clark, fait remarquer que l'on désigne sous le nom de *tractus intestinalis* tout le tube digestif et que l'on devrait, par conséquent, remplacer *equi* par *intestinalis*. On peut ajouter que le mot *equi* n'est pas plus exempt de critiques que l'autre puisqu'il y a plusieurs espèces de Gastrophiles qui s'attaquent au Cheval.

Pour ces raisons et afin de me conformer aux règles de la nomenclature établie il y a plusieurs années, je rétablirai le mot *intestinalis*, qui est le premier en date, pour désigner l'espèce de Gastrophile la plus fréquente du Cheval.

L'Insecte parfait est une Mouche velue, couleur de rouille et tachetée de brun, dont les ailes transparentes, sans nervure transversale terminale, présentent dans le milieu une bande transversale enfumée et deux points de même teinte à leur extrémité libre. Les trochanters des pattes postérieures portent un crochet courbe chez le mâle, un tubercule chez la femelle.

On la trouve dans toute l'Europe, en Afrique, en Asie et dans l'Amérique du Nord.

Dans le courant de l'été, principalement au moment des plus fortes chaleurs, la femelle voltige, en bourdonnant, autour des Chevaux, des Anes ou des Mulets et, sans se poser, colle ses œufs aux poils des régions accessibles à la langue. L'animal, en se léchant, introduit les larves dans sa bouche d'où elles sont ensuite emportées dans l'estomac avec les aliments. Arrivées là, elles se fixent de préférence à la muqueuse du sac gauche. Au bout d'une dizaine de mois, après avoir subi plusieurs mues, elles ont atteint leur complet développement ; elles prennent alors une teinte couleur chair, se détachent spontanément de la paroi stomacale et, sans s'arrêter dans l'intestin, sont expulsées avec les matières fécales. Elles se cachent ensuite dans ces matières ou s'enfoncent dans la terre ; elles deviennent raides, dures, immobiles et leur couleur passe au noir. Au bout de 4 à 6 jours la transformation de la larve en *pupe* est effectuée. C'est une pupe en barillet dans laquelle la *nymphe* reste enfermée pendant 30 ou 40 jours. Il en sort, au bout de ce temps, l'Insecte parfait qui nous a servi de point de départ.

Ces animaux ne sont nuisibles qu'à l'état de larves, et seulement durant leur séjour dans l'estomac. Les effets qu'elles produisent sur les Chevaux qui en sont atteints ont été interprétés de bien des manières par les auteurs. Les uns ont prétendu que les larves finissent par perforer les tuniques de l'estomac et amener la mort ; d'autres, avec Bracy-Clark (**5**), affirment au contraire que, loin de nuire, elles ont une influence favorable sur l'économie en servant de stimulants naturels des fonctions digestives. Des cas de mort survenue à la suite de perforations de l'estomac par les larves gastricoles ont été signalés d'une façon certaine par Numan (**28**) ; mais un examen attentif lui a démontré que l'organe perforé était toujours atteint d'une affection morbide, grâce à laquelle les larves pénétraient plus profondément dans ses parois. Étant donné que le nombre des cas rapportés par Numan et les auteurs les plus récents constituent une proportion extrêmement minime, eu égard à la quantité de Chevaux infestés de ces parasites, il est tout naturel de penser que, sans être absolument inoffensifs, ils ne produisent, en général, que des dégâts peu considérables. L'étude des lésions habituelles nous montrera qu'il en est ainsi.

Les symptômes qui annoncent la présence des larves sont, d'ailleurs, très obscurs ; ils sont même nuls ou tout-à-fait inappréciables quand elles sont peu nombreuses.

Quant au traitement, il était réduit jusqu'à ces derniers temps aux moyens prophylactiques, consistant en des pansages fréquents et des soins de propreté ayant pour but d'empêcher les jeunes larvules, nouvellement écloses, de pénétrer dans l'estomac. C'est en vain, lorsqu'elles s'y étaient installées, qu'on avait tenté de les éliminer en s'adressant aux purgatifs et aux anthelminthiques les plus énergiques. Des expériences nombreuses instituées dans le but de faire périr les larves extraites de l'estomac, en les plongeant dans des liquides toxiques variés ou dans des gaz irrespirables, ont montré combien est grande leur vitalité. Pour arriver à les tuer à l'intérieur même de l'hôte, il eût fallu se servir de produits que le Cheval n'aurait pu supporter sans danger.

Cependant, il y a quelques années, après avoir remarqué que les larves meurent très vite dans le sulfure de carbone, Perroncito (**29**) a réussi, grâce à cette substance, à débarrasser les Chevaux de leurs dangereux commensaux. A la suite du traitement qui consiste à faire avaler au quadrupède le sulfure de carbone sous forme de capsules, les larves seraient rejetées, privées de vie, avec les excréments.

GASTROPHILUS HÆMORRHOÏDALIS (Leach) Linné.

Synonymie : *Œstrus hæmorrhoïdalis* Linné, 1761 ; *Gastrophilus hæmorrhoïdalis* Leach, 1817 ; *Gastrus hæmorrhoïdalis* Meigen, 1824.

A l'état parfait, l'Insecte est très velu, à ailes transparentes et comme enfumées, mais sans taches.

Espèce observée dans toute l'Europe. Après le *G. intestinalis* (Degeer) Leach, c'est l'espèce la plus répandue en France.

Elle a une évolution à peu près identique à celle de l'espèce précédente. D'après Bracy-Clark (**5**), la femelle déposerait sur les lèvres et les poils des lèvres du Cheval de petits œufs noirs et pédicellés. A la maturité il en sort une larve qui gagne, en rampant, la cavité buccale d'où elle est ensuite entraînée dans l'estomac avec les aliments. Les larves, mélangées à celles du *G. intestinalis* (Degeer) Leach, sont d'une couleur rouge plus ou moins intense, plus prononcée à l'extrémité postérieure.

Avant de sortir du tube digestif, elles s'arrêtent quelque temps dans le rectum où elles prennent une teinte verte. Quelquefois elles restent cramponnées à la marge de l'anus, pendant plusieurs heures avant de se laisser tomber à terre.

La transformation en pupe, la durée de la nymphose et l'éclosion de l'Insecte ailé sont identiques à ce que l'on observe chez le Gastrophile précédent.

GASTROPHILUS NASALIS (L.) Schiner

Synonymie : *Œstrus nasalis* Linné, 1761 ; *Œstrus veterinus* Clark, 1797 ; *Œstrus salutiferus* Clark, 1815 ; *Œstrus Clarkii* Leach, 1817 ; *Gastrus nasalis* Meigen, 1824 ; *Œstrus duodenalis* Schwab, 1840 ; *Gastrophilus nasalis* Schiner, 1861.

Espèce ayant une teinte générale noire, recouverte de poils nombreux et fins. Les ailes, assez petites, sont transparentes et blanchâtres.

On la rencontre en Europe et dans l'Amérique du Nord.

On ne sait pas exactement où la femelle pond ses œufs. Numan (**28**) croit que c'est sur les ailes du nez et les lèvres du Cheval, tandis que pour Brauer (**6**) la ponte aurait lieu dans la région de la gorge.

Les larves s'observent à peu près exclusivement dans le duodenum du Cheval, entassées au voisinage du pylore. Lorsqu'elles sont mûres, elles ont une couleur blanc jaune cire et sont rejetées avec les excréments sans jamais se fixer à la marge de l'anus.

La nymphose n'offre rien de particulier.

ŒUFS DE *Gastrophilus intestinalis* (DEGEER) LEACH.

DESCRIPTION. — L'œuf de *Gastrophilus intestinalis* a la forme d'un cône comprimé dont la base, ou pôle antérieur, obliquement tronquée, est munie d'un opercule ovalaire, convexe extérieurement et entouré d'un rebord ; le sommet, ou pôle antérieur est pointu ; toute la surface est finement striée transversalement. Il est d'une couleur jaune blanchâtre. Sa longueur est de $1^{mm}28$ et sa largeur, à la base, de $0^{mm}35$.

Les œufs sont placés sur les poils à la façon des lentes de poux ; ils leur adhèrent par la moitié de leur longueur, à partir du sommet, de telle sorte que la base, dirigée vers le bas, reste libre et écartée du poil.

Adhérence aux poils. — Les œufs sont retenus très solidement aux poils au moyen d'une matière glutineuse émise par la femelle au moment de la ponte. Pour les en séparer, il est nécessaire d'exercer un effort assez grand et, très souvent, on n'arrive qu'à détériorer l'œuf. Dans le but de m'assurer si cette substance agglutinante était soluble dans l'eau, j'ai laissé séjourner, durant plusieurs heures, poils et œufs dans l'eau froide, sans aucun résultat. Maintenus pendant quelques minutes dans l'eau bouillante, les œufs restaient aussi intimement adhérents qu'avant l'expérience.

Fréquence. — On sait que la femelle, guidée par un instinct particulier, dépose ses œufs sur les parties du corps que la langue peut atteindre, et que l'endroit de prédilection est la face interne des genoux.

J'ai remarqué que les Chevaux infestés de ces parasites sont très nombreux, surtout les Chevaux de la campagne. S'ils en ont presque tous, la Mouche s'attaque néanmoins, de préférence, à ceux qui ont une robe de couleur; sur les Chevaux blancs on en trouve très peu ou pas du tout. Pour les apercevoir sur ces derniers, il est nécessaire de s'approcher de très près du quadrupède à cause de la couleur blanc jaunâtre des œufs; sur les autres, on les distingue facilement à plusieurs mètres de distance, accumulés à la face interne des genoux et tout le long du canon, jusqu'au sabot.

Il est extrêmement commun de rencontrer les œufs ailleurs qu'aux genoux; on les trouve souvent à l'extrémité libre des poils de la crinière, derrière les épaules et sur les flancs, mais alors ils sont très clairsemés. Les larves n'en sont pas pour cela destinées à périr; il est, en effet, fréquent de voir, dans les pâturages, les Chevaux se lécher mutuellement. D'autre part, il est possible, selon Numan (**28**), que la larve rampe sur la peau à l'aide de ses crochets et arrive ainsi à s'introduire dans la bouche du Cheval. D'après mes constatations, une jeune larve venant d'éclore, déposée sur la main préalablement humectée d'un peu de salive, parcourt deux centimètres environ à la minute, lorsqu'elle marche en ligne droite. Mais comme il lui arrive souvent de changer de direction et même de revenir sur ses pas, elle évolue en somme dans un espace très restreint. Il en est sans doute de même lorsqu'elle se meut entre les poils du Cheval; dans ce cas elle risque fort de périr avant d'atteindre la bouche. Il me semble qu'il y en a bien peu qui puissent arriver à pénétrer de cette façon dans le tube digestif. Je crois plutôt que ces larves sont, pour la plupart, destinées à manquer leur but et que, seuls, les œufs des régions accessibles à la langue sont capables de laisser échapper des larves qui s'introduiront dans la cavité buccale, lorsque le Cheval les fera éclore par le contact de sa langue humide et chaude. La Nature paraît d'ailleurs avoir prévu le cas en accordant aux femelles une très grande fécondité.

Éclosion. — Tant que dure le développement de l'embryon, l'opercule adhère assez fortement à l'œuf ; quand l'éclosion est sur le point d'avoir lieu, il se détache au contraire avec facilité ; aussi la larve n'a-t-elle que peu d'efforts à exercer pour le faire tomber entièrement.

Pour s'assurer de la maturité de l'œuf, Numan (**28**) l'humectait de salive et lui communiquait la chaleur de la main en le roulant entre les doigts ; la coquille éclate et la larve en sort. Cette expérience, à laquelle on soumet les poils portant des œufs, ne réussit pas si ces derniers ne sont pas arrivés à maturité.

J'ai constaté que, lorsque l'œuf est bien mûr, il suffit de le déposer simplement sur la main pour que la larve fasse immédiatement sauter l'opercule et se mette à ramper avec agilité. Si, quoique mûr, il ne s'ouvre pas, un excellent procédé qui réussit toujours consiste à souffler avec précaution entre les deux mains appliquées l'une contre l'autre; l'haleine humide ainsi projetée sur l'œuf le fait éclore aussitôt. En agissant de la sorte on ne fait qu'imiter les Chevaux; ceux-ci font, en effet, éclore les œufs en leur communiquant la chaleur humide de la langue, lorsqu'ils se lèchent.

Durée de l'incubation. — On ignore le temps qui s'écoule entre le moment de la fécondation et celui de la ponte; on ne connaît pas davantage celui qu'un œuf pondu exige pour arriver à maturité. Bracy-Clark admet que 4 ou 5 jours suffisent pour le développecomplet de l'embryon. Joly (**16**, p. 187) contredit formellement cette assertion en se basant sur ce fait qu'ayant placé des œufs recueillis aux poils d'un Cheval, les uns dans un flacon humide et les autres à sec dans une boîte de carton, il a vu les premiers éclore au bout de 23 jours et les autres 2 ou 3 jours plus tard. A la suite de ces expériences et d'autres analogues, il conclut en ces termes : « il faut au moins 20 ou 25 jours pour que les œufs éclosent, *lorsqu'ils ont été séparés du Cheval qui les portait.* Un peu moins de temps suffit peut-être si on les laisse sur l'animal ; mais, même dans ce dernier cas, nous sommes disposé à croire que l'évolution du germe ne peut jamais s'opérer en cinq jours ».

Les conclusions de Joly ne peuvent être prises en sérieuse considération ; il ne dit pas, en effet, si ces expériences portaient sur des œufs venant d'être pondus ou sur des œufs fixés depuis un certain temps aux poils du Cheval. C'est que, sur le même animal, on trouve à la fois, le même jour, des œufs sans doute fraîchement pondus, puisqu'ils renferment une masse fluide blanchâtre, et des œufs mûrs, que l'on fait éclore au moyen du procédé indiqué tout à l'heure. Je me suis assuré du fait dans maintes circonstances; toutes les fois que j'ai pris des œufs sur les poils des Chevaux, j'ai constaté qu'au moment même on pouvait faire sortir des larves de quelques-uns d'entre eux.

Désirant savoir combien de temps après leur séparation de l'hôte les œufs contenaient encore des larves vivantes, je mis en observation ceux que j'avais recueillis le 22 septembre 1900. Je plaçai les poils couverts d'œufs dans des cornets de papier, après avoir fait des lots séparés, chaque lot correspondant à un seul cheval, et je mis tous les paquets dans une boîte de carton, à la température de l'appartement.

De temps en temps je m'assurais qu'il en sortait des larves vivantes. Chaque fois, je pouvais constater que quelques œufs étaient éclos spontanément et avaient laissé sortir des larves que je retrouvais complètement desséchées.

Le 28 décembre 1900, c'est-à-dire 3 mois et 6 jours après la récolte des œufs, j'obtenais encore des larves bien agiles, en plaçant simplement les œufs dans la main. Je restai ensuite jusqu'au 7 janvier suivant sans pouvoir les observer ; mais à cette date, malgré toutes mes tentatives, je ne pus faire éclore un seul œuf. La température extérieure ayant considérablement baissé pendant cet intervalle de temps et par suite aussi celle de l'appartement non chauffé où se trouvaient les œufs, je me suis demandé si le froid n'avait pas contribué à faire périr les larves dans l'œuf. Je suis porté à admettre qu'il n'en est rien ; j'ai réussi, en effet, le 7 janvier 1901 et jours suivants, jusqu'au 13 inclus, à faire éclore des œufs pris sur des Chevaux le 6 octobre de l'année précédente. Or, ils avaient été soumis aux mêmes changements de température que les premiers.

Comme il est peu probable que les œufs mettent 3 mois et 6 jours pour arriver à maturité, à partir du moment où ils ont été pondus, il est donc extrêmement curieux de voir ces petits êtres, en apparence si fragiles, rester en quelque sorte à l'état de vie latente pendant un intervalle de temps aussi considérable.

Cette expérience prouve que pour avoir une idée de la durée d'incubation des œufs, il est nécessaire, abstraction faite du temps qui s'écoule entre le moment de la fécondation et celui de la ponte, d'assister à la ponte, de recueillir les œufs pondus et de les suivre dans leur développement ultérieur.

Il doit être relativement facile d'avoir des œufs fraîchement pondus, si j'en juge par ce fait que j'ai pu m'emparer, le 6 octobre dernier, d'une femelle de *Gastrophilus intestinalis* occupée à déposer ses œufs sur les poils d'un Cheval, dans l'enceinte même de la ville de Vannes. Le même phénomène doit s'observer encore bien mieux en pleine campagne, au moment des fortes chaleurs, alors que ces Insectes se livrent avec ardeur à la reproduction.

Lorsque l'hiver approche, les larves renfermées dans les œufs qui sont restés adhérents à l'hôte doivent évidemment se ressentir du froid et mourir si celui-ci devient trop vif. J'ai toutefois observé qu'en octobre et en novembre il est possible de faire éclore des œufs pris sur des Chevaux en plein air. J'ai même assisté, le 1er décembre 1900, à l'éclosion d'un œuf dans ces conditions : au contact des doigts il en sortit aussitôt une jeune larvule bien vivante. Je dois ajouter qu'à cette date le froid ne s'était pas encore fait sentir et que, pendant les mois précédents, le temps était resté très doux et très humide.

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LARVE DE *Gastrophilus intestinalis* (DEGEER) LEACH AU PREMIER STADE

A cause, sans doute, de leur fréquence dans l'estomac et de la facilité de se les procurer, les larves adultes seules ont été à peu près exclusivement l'objet de l'attention des vétérinaires et des naturalistes. Les jeunes larves, au contraire, de très petite taille et se prêtant par suite plus difficilement à l'examen à l'œil nu, ont été plus délaissées. Rien de plus facile, cependant, que de se les procurer, en plus grande quantité même que les larves adultes, puisqu'il suffit d'approcher les Chevaux dans le courant de l'été, pour faire une abondante provision d'œufs et, par là même, de petites larvules.

Avant le mémoire de Joly, il n'est question des jeunes larves que pour en signaler l'existence. Numan (**28**), cependant, y découvre les germes des couronnes d'épines et les crochets de la tête. Dans le travail qu'il publia en 1846, Joly (**16**, p. 188) décrit la larvule de la façon suivante : « Au moment de sa naissance, la larvule a une forme et même une structure assez différentes de celles que nous verrons chez la larve parvenue au terme de son accroissement. En effet, au lieu d'être à peu près conique et ramassée comme cette dernière, elle est presque fusiforme, très allongée et formée de treize segments, dont les premiers sont très difficiles à compter. Dans l'état de repos, les deux ou trois premiers segments sont invaginés l'un dans l'autre, ce qui rend la supputation des anneaux beaucoup plus difficile et quelquefois même entièrement impossible.

Le segment céphalique, outre deux prolongements charnus et rétractiles marqués d'un point noir à leur extrémité (palpes ou antennes ?), porte encore deux crochets (mandibules) qui servent à la jeune larve, une fois introduite dans l'estomac, à s'attacher à la membrane interne de cet organe. Une couronne et un faisceau d'épines presque aussi grandes que les crochets, mobiles, recourbées comme eux, et placées tout à fait en avant du deuxième anneau, concourent également à fixer l'animal, rappelant ainsi jusqu'à un certain point l'organisation de plusieurs vers intestinaux et notamment celle des *Echinorhynques*.

Des épines analogues aux précédentes garnissent les bords postérieurs de chacun des neuf anneaux qui suivent la tête : le dixième, le onzième et le douzième en sont complètement dépourvus. Ce dernier offre à son extrémité libre deux espèces de lèvres, entre lesquelles on voit sortir de temps en temps deux tubes rétractiles destinés à remplir un rôle important dans l'acte de la respiration.»

Il dit aussi qu'à sa sortie de l'œuf, la larve est couverte à sa partie antérieure d'une portion de la membrane vitelline dont elle ne tarde pas à se débarrasser.

Cette description de Joly ne concorde pas avec les figures qu'il a dessinées. Alors qu'il admet que les épines sont placées au bord postérieur des anneaux, il semble qu'elles soient plutôt représentées au bord antérieur, tout au moins aux premiers anneaux.

Frappé de ce défaut de similitude entre le texte et les figures, persuadé, ainsi que le fait remarquer Brauer (**6**), que les épines doivent être placées, comme chez la larve adulte, au bord antérieur des anneaux, j'entrepris d'élucider cette question. Je ne tardai pas à m'apercevoir qu'il y avait, en effet, erreur et qu'en outre un grand nombre de détails n'avaient pas été indiqués. Je me mis alors en devoir de représenter la larve d'une façon aussi exacte que possible, afin de pouvoir en faire une description plus complète.

Pressé par le temps je ne me suis occupé que des caractères extérieurs ; je laisse à dessein de côté, du moins pour le moment, ce qui se rapporte à l'anatomie interne.

MOYEN EMPLOYÉ POUR EXAMINER LA LARVE. — L'animal est si petit lorsqu'il quitte sa prison, qu'il est absolument nécessaire, pour se rendre compte de sa constitution, de se servir du microscope. On l'observe dans une goutte d'eau, entre lame et lamelle, en ayant soin d'interposer entre elles de petites cales de papier. Il est extrêmement intéressant de suivre cette petite bête dans ses évolutions. Malgré la surface unie du verre, elle réussit, à l'aide de ses crochets, à ramper sur la lame porte-objet par une série de contractions successives de son corps, pendant lesquelles les anneaux se pénètrent les uns les autres à la manière des tubes d'un télescope. Si l'on a soin de renouveler l'eau sous la lamelle, on arrive, sans autre précaution, à conserver la larve vivante pendant vingt-quatre heures et même davantage.

Le déplacement incessant de l'animal ne permettant pas de le dessiner ni de l'examiner à un fort grossissement, on l'immobilise en faisant une autre préparation, sans interposition de cales de papier. Les seuls mouvements qu'elle puisse effectuer dans ces conditions sont des mouvements de latéralité de la partie postérieure de son corps. Par suite de la compression exercée par le poids de la lamelle, les deux faces de la larve se rapprochent l'une de l'autre, l'air contenu dans les trachées est expulsé et la transparence du corps devient telle que l'on aperçoit à la fois les épines de la face ventrale et celles de la face dorsale.

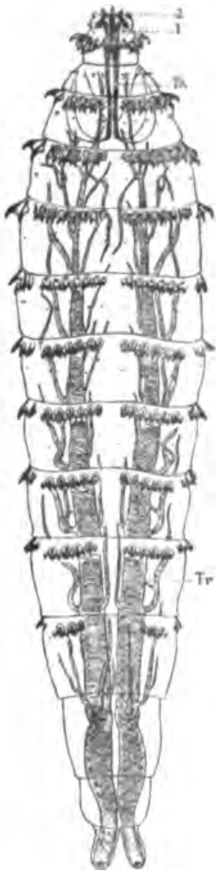

Fig. 1. — Larve de *Gastrophilus intestinalis* (Degeer) Leach au 1er stade, vue par la face dorsale. — *a*, organe tactile ; *l*, antenne ; *Ph*, squelette du pharynx ; *Tr*, trachée. × 80.

Cette disposition ne permet pas de conserver la larve longtemps en vie ; au bout d'une demi-heure à une heure, les anneaux commencent à se déformer, ils se gonflent et la mort survient. Pour l'avoir non déformée, on remplace l'eau par une solution de potasse très concentrée, la glycérine ou le baume de Canada. Au bout de quelques secondes l'animal cesse tout mouvement dans le baume ; mais dans la potasse, il peut résister plus d'une demi-heure, et dans la glycérine, environ une heure et demie. Si l'on désire une préparation immédiate, il est donc nécessaire de tuer la larve ; pour cela, on la plonge dans l'ammoniaque, le chloroforme ou le xylol. Dans les deux premières substances la vie cesse instantanément, tandis qu'avec la seconde, la mort n'arrive qu'au bout de quelques secondes. On monte ensuite l'animal dans la glycérine ou dans le baume. Si l'on emploie le baume, on tue la larve à l'aide du chloroforme ou du xylol ; ces substances enlèvent l'humidité qui recouvre la surface du corps et empêchent ainsi de se produire le trouble que l'on observe dans le baume, sans cette précaution.

Description de la larve a sa sortie de l'œuf. — J'ai examiné beaucoup de larves au moment de leur éclosion, je ne les ai jamais vues recouvertes de la membrane vitelline dont parle Joly.

A ce moment, la jeune larve est allongée, fusiforme, transparente, bifurquée à son extrémité postérieure ; elle offre à la surface des stries noires transversales et espacées dues aux épines qui entourent le corps de l'animal. Elle mesure $1^{mm}25$ de longueur sur $0^{mm}30$ de largeur, au niveau des quatrième et cinquième anneaux.

On peut lui considérer une face supérieure ou dorsale (fig. 1), une face inférieure ou ventrale (fig. 2), celle sur laquelle rampe l'animal, et deux faces latérales.

Le corps est divisé en 12 segments par des sillons transversaux placés immédiatement en avant des couronnes d'épines. A l'extrémité antérieure, si la larvule est bien étalée, on distingue un renflement couvert de nombreuses épines disposées sur plusieurs rangées et diminuant de taille d'avant en arrière ; c'est la première couronne d'épines.

Viennent ensuite 9 autres couronnes placées non pas, comme le dit Joly, au bord postérieur des anneaux, mais bien, ainsi que le suppose Brauer, à leur partie antérieure. Quoique le dessin de Joly soit assez imparfait, on y voit néanmoins que les épines sont figurées

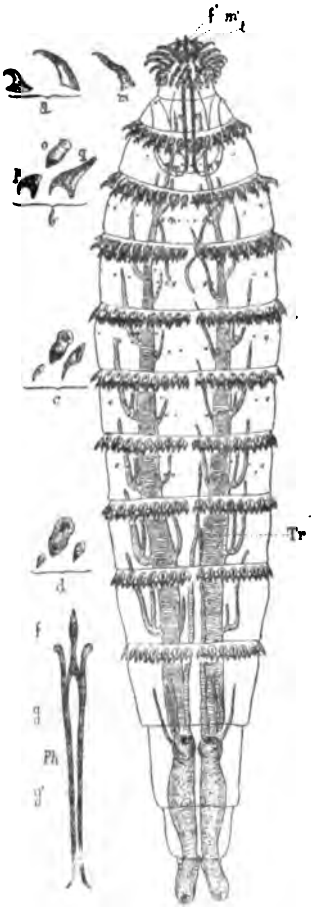

Fig. 2. — Larve de *Gastrophilus intestinalis* (Degeer) Leach au premier stade, vue par la face ventrale. × 80 — *a*, deux crochets de la couronne céphalique, × 230 ; *b*, épines des anneaux 2, 3 et 4, × 230 : *c*, épines des anneaux 5 et 6, × 230 ; *d*, épines des anneaux 7, 8, 9 et 10, × 230 ; *f*, *f'*, stylet ; *g*, *g'*, lames chitineuses du pharynx, × 185 ; *m*, crochet mandibulaire, × 230 ; *m'*, le même, en place ; *o*, une des épines de la première rangée, × 230 ; *p*, crochet de la première rangée, × 230 ; *q*, crochet de la deuxième rangée, × 230 ; *t*, organe tactile ; *Tr*, trachée.

au bord antérieur des anneaux, disposition que l'on retrouve chez la larve adulte. Les couronnes sont interrompues latéralement et sur le milieu des faces dorsale et ventrale, de sorte qu'on a quatre bandes claires longitudinales rappelant les champs latéraux des Nématodes. La bande ventrale est plus large que les autres.

Après le dernier anneau porteur d'épines, on ne trouve pas les trois anneaux de Joly, mais seulement deux segments, le segment terminal étant limité à sa partie postérieure par deux lèvres entre lesquelles on voit sortir deux tubes rapprochés à la base et séparés au sommet. C'est à tort, je crois, que Joly les qualifie de rétractiles. Le dernier anneau, lorsque l'animal se contracte, entre bien dans l'avant-dernier, mais les deux tubes en question ne pénètrent pas dans le dernier segment dont ils ne paraissent être que des prolongements.

Nombre de segments. — Si l'on adopte la même façon de compter que pour la larve au dernier stade, on trouve exactement le même nombre de segments dans les deux cas, c'est-à-dire douze. Seulement, chez la larve adulte, le douzième segment, peu visible extérieurement, est représenté par les deux lèvres vésiculeuses situées à l'arrière du onzième, ce qui réduit leur nombre à onze. Pour faire voir qu'il y a similitude entre les deux stades, il n'y a qu'à couper en deux le tégument de la larve mûre par un plan qui sépare la face ventrale de la face dorsale, à étaler les deux moitiés sur une lame de verre et à les examiner à un faible grossissement ; la ressemblance alors s'impose.

Anneau céphalique (fig. 1 et 2).—Le premier segment de la larvule, ou anneau céphalique, comprend tout ce qui est situé en avant de la deuxième couronne d'épines. On y distingue une partie antérieure, celle qui est placée immédiatement en avant de la première couronne d'épines, et une partie postérieure. Celle-ci est elle-même divisée en deux par un sillon transversal très net qui correspond à la constriction placée au même endroit chez la larve à son complet développement.

La partie antérieure porte les deux antennes (fig. 1, *l.*), se touchant par leur base élargie, séparées au sommet où l'on aperçoit un petit organe jaune (fig. 1, *a*), lequel a la forme d'un bouton relié par un pédicule à l'extrémité de l'antenne. Les antennes sont protractiles et rétractiles. Le point jaune qui les surmonte ressemble plutôt à un organe du tact qu'à un œil. Je ne me rends d'ailleurs pas bien compte de l'utilité des yeux chez des animaux destinés à passer toute leur vie dans une cavité où ne pénètre jamais un rayon lumineux.

A la base des deux lobes antennaires, du côté ventral, on aperçoit les deux crochets buccaux (fig. 2, *m*, *m'*) et, entre eux, un stylet pointu (fig. 2, *f*). Ce dernier, nettement dessiné lorsque la larve fait saillir la partie antérieure de la tête, n'est qu'une partie du squelette du pharynx contenu dans le premier et le deuxième anneau. Le squelette en question est formé de deux lames longitudinales (fig. 2, *g*, *g'*), amincies en arrière et légèrement recourbées en dehors; à leur extrémité antérieure renflée, elles s'articulent avec les deux crochets mandibulaires. Entre les deux lames se place le stylet (fig. 2, *f*), lequel est divisé postérieurement en deux branches qui vont se fixer sur elles.

Si l'on compare cette disposition à ce que l'on trouve chez la larve plus âgée, il est peut-être permis de considérer les crochets mandibulaires de la jeune larve comme étant les homologues de celles de l'adulte; le stylet médian correspondrait alors aux deux mâchoires. Quant aux lames chitineuses longitudinales du pharynx, elles sont identiques dans les deux cas.

Immédiatement en arrière de la partie antérieure du segment céphalique vient un renflement presque sphérique, recouvert d'un grand nombre de crochets chitineux disposés sur plusieurs rangées. Ces crochets sont plus longs et plus nombreux à la face ventrale qu'à la face dorsale et les dernières rangées sont, en outre, interrompues au milieu, dorsalement. Il y a environ 5 rangées à la face inférieure et 2 ou 3 seulement à la face supérieure. La première rangée forme une couronne complète; elle présente cette particularité remarquable que les huit crochets médians de la face ventrale (fig. 2, *a*) sont beaucoup plus longs, moins recourbés et plus mobiles que les autres. Lorsque la larve se déplace, elle s'accroche d'abord à l'aide de ces épines spéciales, puis elle tire après elle le reste de son corps, sans préjudice des autres couronnes d'épines qui jouent également un rôle important dans la locomotion.

Les crochets de la tête (fig. 2, *a*) se composent d'une base élargie, implantée dans le tégument, et d'une partie conique à pointe acérée et fortement recourbée. Les longues épines mobiles paraissent dépourvues de base élargie, mais la partie conique est très grande et assez faiblement concave ; leur taille dépasse légèrement celle des crochets mandibulaires.

Il n'y a pas de doute que cette couronne de tubercules épineux ne représente celle que l'on observe chez la larve au dernier stade ; seulement, les épines sont, comparativement à celles des anneaux suivants, beaucoup plus développées au premier stade qu'au dernier.

Le renflement céphalique se continue par une partie qui se rétrécit graduellement pour se terminer au sillon séparant en deux la partie postérieure du premier anneau ; le segment s'élargit ensuite peu à peu jusqu'à l'anneau suivant.

Telle est la configuration du premier anneau sur l'animal bien étalé ; il est absolument semblable, sauf en ce qui concerne la taille et la forme des crochets épineux, à ce qui existe chez la larve adulte. Il est très difficile de reconnaître ces différentes parties sur le vivant à cause des mouvements continuels d'invagination et de dévagination qu'effectue la larve. Pendant l'invagination tout se confond, mais au moment de l'évagination toutes les parties de la tête se distinguent facilement ; c'est alors que l'on voit le squelette pharyngien projeté en avant, les deux lames chitineuses poussant devant elles les deux mandibules dont les pointes s'écartent en dehors, pendant que le stylet médian fait saillie droit devant lui.

La difficulté n'existe pas pour les anneaux suivants qui demeurent intacts et toujours nettement visibles. .

Anneaux suivants pourvus d'épines. — De tous les anneaux qui suivent la tête, le second est le plus court. Du troisième au dixième ils vont légèrement en augmentant de longueur pour diminuer ensuite jusqu'au douzième.

La largeur augmente du 1^er^ au 4^e^ anneau. C'est à ce niveau que la larve atteint sa plus grande largeur ; elle la conserve à l'anneau suivant, puis cette largeur diminue ensuite progressivement jusqu'au dernier.

A la partie antérieure des anneaux, du 2^e^ au 10^e^ inclusivement, il existe une couronne d'épines qui fait tout le tour du corps. Chaque couronne est interrompue sur les côtés, et un peu dorsalement, par un petit espace dont la largeur est la même à tous les anneaux.

Il y a également une interruption sur la ligne médiane ventrale, mais à partir du 3e anneau seulement. Au milieu de la face dorsale, l'espace nu s'observe à toutes les couronnes d'épines ; il est plus visible qu'à la face opposée et d'autant plus large que l'anneau est plus postérieur.

Répartition des épines. — Chaque couronne, ou cercle, est formée de deux rangées d'épines alternes, dont le nombre, à chaque rangée, va en diminuant d'avant en arrière, aussi bien à la face inférieure qu'à la face dorsale ; mais du côté ventral on observe toujours, sur le même anneau, quelques-unes de plus qu'à la face dorsale. En outre, il est fréquent de trouver une épine de plus d'un côté de la ligne médiane que de l'autre. Sur la larve représentée dans les figures 1 et 2 on compte à chaque rangée :

au 5e anneau (le plus large)	face dorsale,	7	épines d'un côté	de la ligne médiane,	8	de l'autre.
	face ventrale,	10	—	—	9	—
au 10e anneau	face dorsale,	3	—	—	3	—
	face ventrale,	6	—	—	6	—

Forme des épines. — Les épines ont des formes différentes, selon la rangée que l'on considère, à un anneau déterminé, et aussi suivant le rang de l'anneau. Ayant déjà décrit celles de la tête, je n'y reviens pas.

Examinés en place, sur la larve même, les crochets des anneaux 2, 3 et 4 (fig. 2, *b*) semblent, au premier abord, très différents de ceux des anneaux 5 et 6 (fig. 2, *c*) qui eux-mêmes ne paraissent pas semblables aux épines des anneaux suivants 7, 8, 9 et 10 (fig. 2, *d*). En réalité, une observation attentive montre qu'il y a passage d'une catégorie à l'autre. Je considère comme étant la première rangée celle dont la base des épines est située le plus près du sillon de séparation des anneaux.

Épines des anneaux 2, 3 et 4. — Les épines de la première rangée sont formées de deux pièces (fig. 2, *b*) disposées, sur la larve, de telle sorte que l'une d'elles (fig. 2, *p*) paraît être une dépendance de l'autre (fig. 2, *o*). La première est un crochet chitineux semblable à ceux de la tête, à base large, implantée dans le tégument, à pointe très acérée et fortement recourbée. La seconde a la forme d'un petit cylindre aplati, étranglé vers son milieu et terminé en pointe à l'extrémité postérieure. Elle recouvre le crochet de telle façon que ce dernier ressemble à une petite pince placée à l'extrémité de la pièce cylindrique. On n'arrive à bien distinguer ces deux parties l'une de l'autre que quand l'animal se contracte ; la seconde pièce se présente alors sous l'aspect d'un clapet qui se rabat sur le crochet sous-jacent.

La deuxième rangée se compose de crochets semblables à celui qui vient d'être décrit (fig. 2, *q*), seulement ils sont beaucoup plus longs. Leur pointe est dirigée en arrière et fortement recourbée comme les premiers.

Ces trois sortes d'épines ne sont autre chose que des tubercules de forme particulière, faisant saillie à la surface du revêtement de chitine qui entoure le corps de la larve.

Epines des anneaux 5 et 6. — Aux deux anneaux suivants, 5 et 6, on retrouve les mêmes tubercules épineux (fig. 2, *c*) ; ils diffèrent de ceux du premier groupe en ce que l'une des pièces (fig. 2, *o*) a augmenté de taille alors que les autres, les crochets (fig. 2, *p*, *q*), ont diminué de longueur et sont moins recourbés.

Epines des anneaux 7, 8, 9 et 10. — Dans ce groupe (fig. 2, *d*), la différence que je viens de signaler entre les deux premiers est encore plus accentuée. L'opercule (fig. 2, *o*) est devenu très grand, tandis que les crochets (fig. 2, *p*, *q*), très réduits, ont pris l'aspect de petits cônes chitineux, à pointe droite ou très faiblement recourbée.

Anneaux entièrement dépourvus d'épines. — Les anneaux 11 et 12 sont complètement nus. Le dernier se termine, comme nous le savons, par deux lèvres entre lesquelles passent les deux prolongements qui terminent les trachées. La lèvre ventrale présente au milieu de son bord postérieur une petite fente longitudinale dont les bords portent de chaque côté un filament chitineux fin et court. Les deux filaments sont dirigés en arrière et convergent l'un vers l'autre.

Les tubes du dernier anneau offrent à leur base des rides transversales permanentes ; à l'extrémité libre de chacun d'eux on aperçoit l'orifice stigmatique.

Ornements spéciaux. — La surface antérieure du corps, principalement la face inférieure, est recouverte de petites productions chitineuses que je signale à cause de la régularité de leur disposition. Au milieu de la dernière partie de l'anneau céphalique, ainsi qu'au milieu des deux anneaux suivants, un peu en dehors de la ligne médiane ventrale, on distingue, de chaque côté, un groupe de trois petites aiguilles chitineuses qui partent du même endroit et dont la pointe libre est dirigée en arrière. De plus, il existe aux sept premiers anneaux des pointes chitineuses très petites, ayant l'apparence de boucles de raie, disposées symétriquement de chaque côté de la ligne médiane.

Trachées. — Sans entrer dans l'anatomie des organes internes, je ferai toutefois remarquer que la transparence du tégument laisse voir deux énormes trachées (fig. 2, *Tr*) qui courent tout le long du corps. L'air qui a pénétré par les deux stigmates postérieurs séjourne dans les renflements des deux derniers anneaux et de là se répand dans les grosses trachées. Celles-ci diminuent graduellement de diamètre d'arrière en avant, émettant tout le long de leur trajet des ramifications latérales symétriquement disposées. Ces ramifications se divisent à leur tour en trachées de plus en plus fines qui finissent par former un réseau inextricable à l'intérieur du corps de la larve.

Au niveau du 1er et du 2^{e} anneau, on distingue assez nettement les contours du pharynx. Les conditions dans lesquelles j'ai fait mes observations ne m'ont pas permis de voir le gros ganglion nerveux que Joly a décrit et figuré.

ACTION D'UN CERTAIN NOMBRE DE LIQUIDES SUR LES LARVES DE *Gastrophilus intestinalis* (DEGEER) LEACH AU PREMIER STADE

Ayant remarqué que les petites larves de *G. intestinalis* (Degeer) Leach prolongeaient leur existence pendant plusieurs heures dans l'eau et dans la glycérine dont je me servais pour les examiner au microscope, me rappelant, d'autre part, les expériences tentées pour essayer de détruire les larves adultes, en les plongeant dans des substances toxiques variées, je me suis livré vis-à-vis de ces jeunes larves à une série de recherches dans le même sens, en les soumettant à l'action de liquides où je les plongeais aussitôt après leur sortie de l'œuf. Mes expériences ont été faites le 21 et le 22 novembre 1900, dans un appartement dont la température se maintenait à peu près uniformément aux environs de 14°. Les larves provenaient d'œufs recueillis sur plusieurs Chevaux le 22 septembre précédent ; ces œufs étaient âgés par conséquent de deux mois.

Lorsque, par des expériences préliminaires, je savais que les larves, plongées dans une substance, devaient périr en quelques minutes ou quelques heures, je les plaçais dans une goutte de cette substance, entre lame et lamelle, maintenues écartées à l'aide de petits morceaux de papier. Je pouvais de cette façon les observer facilement, en ayant soin toutefois de remplacer le liquide à mesure qu'il s'évaporait. Si, au contraire, elles résistaient une demi journée ou plus, j'introduisais le liquide dans de petits tubes où les larves pouvaient séjourner sans craindre la dessiccation. De temps en temps je m'assurais qu'elles étaient vivantes en les plaçant dans la main ou bien je les observais au microscope dans une goutte d'eau tiède.

Le tableau suivant résume les résultats que j'ai obtenus :

SUBSTANCES EMPLOYÉES	TEMPS AU BOUT DUQUEL LES LARVES VIVAIENT ENCORE	TEMPS AU BOUT DUQUEL ELLES AVAIENT CESSÉ DE VIVRE
Eau ordinaire	94 h.	100 h.
Eau distillée	52 h.	56 h.
Solution saturée d'alun dans l'eau	49 h.	61 h.
Huile d'olive	20 h. 1/2	23 h. 1/2
Sublimé au 1000^e	14 h. 1/2	17 h.
Eau de chaux	8 h. 1/2	9 h. 1/2
Solution saturée d'acide borique	5 h. 15'	5 h. 33'
Alcool méthylique	3 h. 1/2	18 h.
Pétrole	1 h. 1/2	3 h.
Glycérine	1 h. 1/2	1 h. 3/4
Alcool à 45°	1 h. 5'	1 h. 15'
Eau de Javelle du commerce étendue de 5 fois son volume d'eau	40 min.	55 min.
Solution très concentrée de potasse dans l'eau	35 min.	51 min.
Solution saturée de sublimé dans l'eau	33 min.	48 min.
Acide azotique à 5 °/o	17 min.	19 min.
Acide chlorhydrique à 5 °/o	17 min.	20 min.
Formol à 5 °/o	10 min.	12 min.
Acide sulfurique à 1 °/o	9 min.	17 min.
Acide acétique glacial à 5 °/o	8 min.	16 min.
Eau de Javelle du commerce	7 min.	8 min.
Formol du commerce pur	»	1 min.
Gaz mill.	30 sec.	1 min.
Xylol pur	»	10 sec.
Essence de térébenthine	mort après quelques mouv.	
Teinture d'iode	id.	
Acide azotique pur	id.	
Acide sulfurique pur	mort instantanée	
Acide chlorhydrique pur	id.	
Acide acétique pur	id.	
Alcool à 90°	id.	
Alcool à 100°	id.	
Sulfure de carbone	id.	
Benzine	id.	
Ammoniaque liquide	id.	
Ether sulfurique	id.	
Chloroforme	id.	

Il résulte de ces expériences que, bien qu'étant d'une structure assez délicate, la jeune larvule fait voir ce qu'elle deviendra plus tard. Elle peut en effet résister plus de quatre jours lorsqu'elle est immergée dans l'eau ordinaire, près d'un jour dans l'huile, plus de quatorze heures dans le sublimé au 1000^e, etc. Le même tableau fournit un renseignement que l'on pourra peut-être utiliser dans la pratique : c'est que la larve succombe instantanément lorsqu'on la plonge dans les liquides volatils. N'est-ce pas, d'ailleurs, au moyen d'une substance toxique volatile, le sulfure de carbone, que Perroncito (**29**) a découvert un remède efficace, capable d'expulser les larves d'Œstres de l'estomac ?

LARVE DE *Gastrophilus intestinalis* (DEGEER) LEACH
A L'AVANT-DERNIER STADE

Contrairement à l'opinion de Bracy Clark qui pensait qu'elles ne sont pas sujettes à la mue, les larves gastricoles doivent se dépouiller plusieurs fois de leur revêtement chitineux avant d'atteindre leur développement complet.

Numan (**28**, p. 344) signale, en effet, parmi les larves rouges de l'Œstre hémorrhoïdal que l'on observe chez les Chevaux, en automne et au commencement de l'hiver, des individus sur lesquels on découvre une triple couronne d'épines.

Ces larves portent, en outre, une double rangée d'épines un peu plus longues que les précédentes et qui, au lieu de s'élever au dessus de la peau, sont cachées et couvertes d'une membrane ou épithélium. Il en conclut qu'à une époque peu avancée de leur développement, elles perdent leur épithélium avec la triple couronne d'épines qui s'y trouvent implantées. Et il ajoute qu'il ignore à quelle espèce ou variété peuvent bien appartenir ces petites larves rouges.

Mes recherches m'ayant mis en présence de larves identiques à celles que mentionne Numan, je me suis proposé de vérifier l'hypothèse qu'il émet au sujet de la mue. Après m'être rendu compte de la réalité du fait, je me suis assuré qu'elles appartenaient à l'espèce *Gastrophilus intestinalis* (Degeer) Leach.

Je les ai trouvées, le 20 novembre 1900, dans un estomac de Cheval; elles étaient au nombre de quatre, mélangées à vingt-deux larves plus âgées de la même espèce, fixées, selon l'habitude, à la muqueuse du sac gauche.

Avant d'en donner la description, je ferai observer qu'elles donnent naissance, après la mue, à une forme qui possède, sauf la taille, tous les caractères de l'adulte. Puisqu'on ne connaît pas encore toutes les phases intermédiaires entre la première et la dernière, j'appellerai cette forme *larve à l'avant-dernier stade.* C'est celle que Brauer (6) désigne sous le nom de larve au deuxième stade.

Description. — Cette larve (fig. 3) a une forme très allongée, plus large en arrière qu'en avant et nettement divisée par des sillons profonds en douze segments. Comme chez l'adulte, le douzième segment est formé de deux lèvres placées à l'arrière du onzième anneau dans lequel elles sont en partie cachées, de sorte qu'il ne paraît y en avoir que onze. Toutefois, les lèvres, moins rentrées que chez la larve au dernier stade, sont légèrement saillantes en arrière. Elles limitent la fente qui donne accès dans la cavité au fond de laquelle sont placées les plaques stigmatiques.

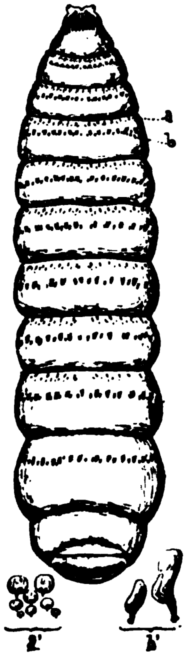

Fig. 3. — Larve de *Gastrophilus intestinalis* (Degeer) Leach à l'avant-dernier stade, vue par la face ventrale, × 4, 5. — *a*, épines de la larve à l'avant-dernier stade ; *a'* portion d'une couronne d'épines de cette larve, × 17 ; *b*, épines de la larve au dernier stade ; *b'*, deux épines de cette larve, × 17.

La longueur totale du corps de la larve que j'ai étudiée était de 17mm et sa largeur de 4mm5 au niveau du dixième anneau, le plus volumineux. Ces dimensions sont considérables parce que j'avais là une larve sur le point de muer, mais j'ai eu l'occasion d'en examiner de plus petites et de plus jeunes sous le tégument desquelles on n'apercevait aucune trace des épines de la forme suivante.

La longueur et la largeur des anneaux augmentent de la tête au dixième anneau qui est à la fois le plus long et le plus large. Ces deux dimensions diminuent brusquement à l'anneau suivant.

La couleur est d'un beau rouge sur toute l'étendue de la surface du corps, sauf à l'extrémité postérieure, où cette teinte est plus foncée.

Anneau céphalique. — Le premier anneau possède exactement la même conformation extérieure que chez l'adulte. Il se compose de deux parties ; la première porte : 1° deux antennes ; 2° deux crochets mandibulaires ; 3° entre eux, deux mâchoires chitineuses. Tout à fait en avant de la deuxième partie, le tégument est couvert de plusieurs rangées de très petites épines. En arrière de ces épines vient une constriction à partir de laquelle l'anneau s'élargit de nouveau. C'est la reproduction en plus petit de ce qui existe chez la larve au dernier stade.

Répartition des épines. — Nous venons de voir que le premier anneau possède une première couronne de petites épines. Au bord antérieur de chacun des anneaux suivants, du deuxième au huitième inclusivement à la face dorsale, du deuxième au neuvième à la face ventrale, on remarque un cercle d'épines semblables disposées suivant plusieurs rangées (fig. 3, *a*). Les cercles, ou couronnes, sont interrompus au milieu des deux faces par un espace nu, très petit du côté ventral, plus large à la face supérieure, surtout aux derniers anneaux. La face dorsale du 8^e anneau ne porte que quelques épines sur les côtés ; les anneaux 10 et 11 sont entièrement nus sur les deux faces.

Les couronnes des premiers anneaux se composent de quatre rangées d'épines alternes et dirigées en arrière ; celles des derniers, de trois seulement.

On remarquera que les épines vont moins loin ici que chez l'adulte où on les trouve au 10^e anneau du côté ventral et au 9^e du côté dorsal. S'il existe une couronne de moins, en revanche, le nombre des rangées d'épines de chacune d'elles est plus grand.

Forme des épines. — Les épines (fig. 3, *a'*), extrêmement fines, à peine perceptibles à l'œil nu, sont formées d'une base élargie, circulaire ou elliptique, adhérente au tégument, et d'un petit aiguillon court, très pointu, placé excentriquement et dirigé en arrière. La base est incolore et transparente tandis que l'aiguillon est jaune brun, plus foncé à son point d'émergence qu'à son extrémité libre. Les épines d'une même couronne sont d'autant plus petites qu'elles appartiennent à une rangée plus postérieure.

Plaques stigmatiques. — La lame stigmatique, très petite, doit être examinée au microscope. Elle est formée de deux lames brunes, réniformes, se regardant par leur bord concave. Chaque lame se compose de deux arcs seulement, alors qu'au dernier stade il y en a trois. Les deux lames sont réunies par un encadrement chitineux très épais.

Lorsque la larve est suffisamment âgée, on voit se dessiner sous sa peau les épines (fig. 3, *b*) qu'elle possèdera au stade suivant. Celles-ci sont appliquées le long du corps, la pointe dirigée en arrière, par la cuticule qui les recouvre. Plus grandes et plus visibles qu'à la phase précédente, elles sont disposées sur deux rangées alternes, suivant des cercles qui semblent placés au milieu des anneaux. Il existe une couronne de plus qu'au stade précédent ; le 10ᵉ anneau possède, en effet, des épines au bord antérieur de sa face ventrale et le 9ᵉ en a du côté dorsal.

Si, au moyen de ciseaux, on débarrasse la nouvelle larve de son ancienne enveloppe, on a devant soi, sauf la forme et les dimensions, une larve au dernier stade. On y retrouve les épines disposées exactement de la même façon et munies à leur extrémité libre d'un renflement caractéristique (fig. 3, *b'*) ; les arcs stigmatiques sont maintenant au nombre de trois à chaque lame. En résumé, il ne lui reste plus désormais qu'à grandir pour acquérir son complet développement.

Pour savoir s'il existe une forme intermédiaire au 1ᵉʳ et à l'avant-dernier stade, il faudrait rencontrer une larve de cette dernière catégorie avec ses triples et quadruples rangées d'épines, recouverte de son ancien tégument, et constater que celui-ci diffère du revêtement si caractéristique de la larve au 1ᵉʳ stade.

En raison de ce fait qu'il existe trois arcs à chaque lame stigmatique chez la larve au dernier stade et deux arcs seulement chez la larve au stade précédent, alors qu'au premier stade il n'y en a pas encore, je suis convaincu qu'il y a une quatrième forme possédant un seul arc de chaque côté à la plaque stigmatique. L'évolution de la larve comprendrait dans ce cas quatre stades séparés par trois mues.

ÉTUDE COMPARATIVE DES LARVES DE *Gastrophilus intestinalis* (DEGEER) LEACH, *G. hæmorrhoïdalis* (L.) LEACH ET *G. nasalis* (L.) SCHINER AU DERNIER STADE

Les larves d'Œstrides que l'on observe dans l'estomac du Cheval, en France, appartiennent toutes aux trois espèces *G. intestinalis* (Degeer) Leach, *G. hæmorroïdalis* (L.) Leach et *G. nasalis* (L.) Schiner. La première, la plus répandue, est celle que l'on désigne ordinairement sous le nom d'Œstre du Cheval, de Gastrophile équin.

Mégnin (**24**) a découvert la larve du *G. pecorum* (Fbr.) Schiner sur des Chevaux russes importés aux environs de Paris ; je ne m'occuperai pas de cette dernière.

La première fois que j'aperçus des larves dans l'estomac du Cheval et que je voulus savoir si elles appartenaient toutes à la même espèce, je restai fort embarrassé. A cause de son unique rangée d'épines, la larve de *G. nasalis* pouvait facilement se différencier des deux autres espèces. Pour distinguer ces deux-ci l'une de l'autre, je devais, d'après les ouvrages que je possédais entre les mains, me baser sur le volume, la forme, la couleur et surtout sur la différence de taille entre les épines des deux rangées du même anneau. Or, tous ces caractères varient avec l'âge. Si les grosses larves appartenaient manifestement à l'espèce *G. intestinalis* (Degeer) Leach, il ne m'était pas permis d'être aussi affirmatif vis-à-vis des formes moins volumineuses; dans ce cas, les épines étaient plus petites et la différence entre les deux rangées devenait moins sensible.

Je fis part de mon embarras à M. Railliet qui eut l'obligeance de me donner, pour servir de termes de comparaison, quelques échantillons, conservés dans l'alcool, de chacune des trois espèces.

L'examen attentif de ces larves m'ayant fait découvrir entre elles des différences suffisantes pour les caractériser à première vue, j'ai pensé qu'une étude comparative de leurs caractères extérieurs pourrait peut-être rendre quelques services au point de vue de la détermination de l'espèce.

CARACTÈRES COMMUNS AUX TROIS ESPÈCES. — La larve adulte possède une forme ovoïde, un peu aplatie ventralement, plus étroite en avant qu'en arrière; l'extrémité postérieure est tronquée suivant un plan perpendiculaire à la direction longitudinale de l'animal; le tégument, formé de chitine, est lisse et rigide.

Le corps paraît formé de 11 anneaux. Le premier, ou anneau céphalique, se compose de deux parties séparées par un sillon. La première, très petite, porte : 1° deux antennes se touchant à leur base et terminées à leur extrémité libre par un petit bouton simulant un œil ; 2° au-dessous, une paire de grands crochets mandibulaires recourbés en dehors et en arrière, articulés avec le squelette du pharynx ; 3° entre ceux-ci, une paire de mâchoires droites dont le bord interne, libre, est grossièrement denté ; elles s'articulent également avec les pièces du pharynx ; le petit orifice buccal est situé entre les deux mâchoires. Le bord antérieur de la deuxième partie de l'anneau céphalique est recouvert de plusieurs rangées de très petites épines.

Cette deuxième partie a la forme d'un tronc de cône, à base postérieure, étranglé vers le milieu. Elle présente de chaque côté un bourrelet longitudinal surmonté d'un sillon qui aboutit aux petits organes, invisibles de l'extérieur, qui terminent les trachées en avant. Ces organes que l'on appelle stigmates antérieurs, bien que d'après Schroeder van der Kolk (**41**) ils ne communiquent pas avec l'extérieur, sont cachés dans la partie dorsale du sillon qui sépare le premier anneau du second.

Les dix anneaux qui suivent l'anneau céphalique sont peu différents les uns des autres : ils présentent latéralement deux bourrelets longitudinaux et sur la plupart d'entre eux, sauf les derniers, il existe une couronne d'épines à pointe dirigée en arrière : ces épines sont implantées au bord antérieur de chacun des segments.

Le 11ᵉ anneau a une forme très caractéristique : il offre en arrière une fente transversale limitée par deux lèvres, une supérieure, l'autre inférieure, mobiles, épaisses et comme vésiculeuses, pouvant se rapprocher de façon à fermer la fente hermétiquement. Cet orifice donne entrée dans une cavité au fond de laquelle est située la lame stigmatique, laquelle se compose de deux plaques réniformes, d'un brun jaunâtre, se regardant par leur bord concave et entourées d'un encadrement chitineux. Chacune des plaques est formée de trois arcs concentriques parcourus par un sillon longitudinal auquel viennent aboutir, de chaque côté, de petites fentes transversales. L'espace médian compris entre les deux plaques présente à son centre une fossette d'où partent des plis rayonnants.

Caractères différentiels. — **1° Larve de *G. intestinalis* (Degeer) Leach** (fig. 4, 5 et 6). — Couleur chair jaunâtre. Longueur du corps, 18 à 22mm ; largeur au septième anneau, 8mm.

La longueur de l'anneau céphalique est plus grande que son diamètre transversal à la base.

Epines du premier anneau très petites, disposées sur 8 rangées environ à la face ventrale et sur 4 seulement à la face dorsale.

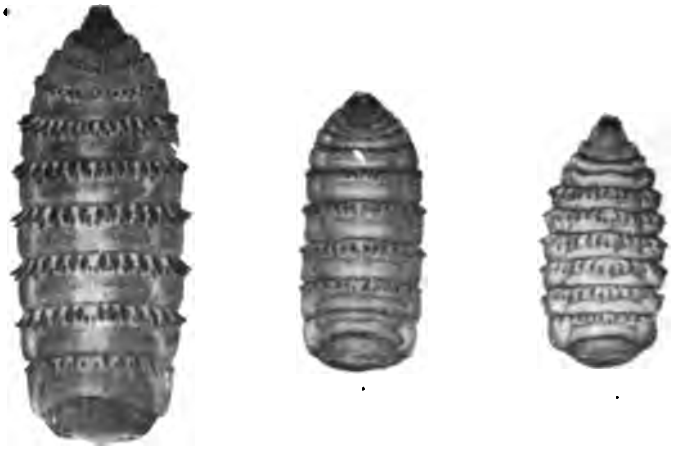

Fig. 4. — Larves de *Gastrophilus* vues par la face ventrale, × 2, 5. — A gauche, larve de *G. intestinalis* (Degeer) Leach ; au milieu, larve de *G. hæmorrhoïdalis* (L.) Leach ; à droite, larve de *G. nasalis* (L.) Schiner.

Du deuxième anneau au dixième inclusivement, à la face inférieure, du deuxième au neuvième à la face supérieure, on aperçoit au bord antérieur des anneaux une double rangée d'épines alternes ; les épines du premier rang sont beaucoup plus grandes que celles du second ; leur disposition est telle que la ligne médiane dorsale passe entre deux épines de la seconde rangée. Chaque couronne est interrompue au milieu du dos par un espace nu qui devient très grand au neuvième anneau. La partie dorsale du dixième anneau paraît complètement nue, malgré une ou deux épines très petites placées de chaque côté. Le onzième anneau est le seul qui soit entièrement dépourvu d'épines.

Les épines sont des tubercules chitineux coniques issus du tégument, jaunâtres à la base, noirs au sommet, dirigés en arrière. L'extrémité libre se termine par un renflement divisé en deux par un sillon transversal (fig. 6). Cette particularité ne se retrouve pas dans les deux autres espèces.

2° **Larve de** *G. hæmorrhoïdalis* (L.) Leach (fig. 4, 5 et 6). — Forme plus allongée et corps moins volumineux que chez le *G. intestinalis* (Degeer) Leach.

Couleur d'un rouge plus ou moins intense, plus prononcé à l'extrémité postérieure. A leur sortie du rectum, les larves sont vertes.

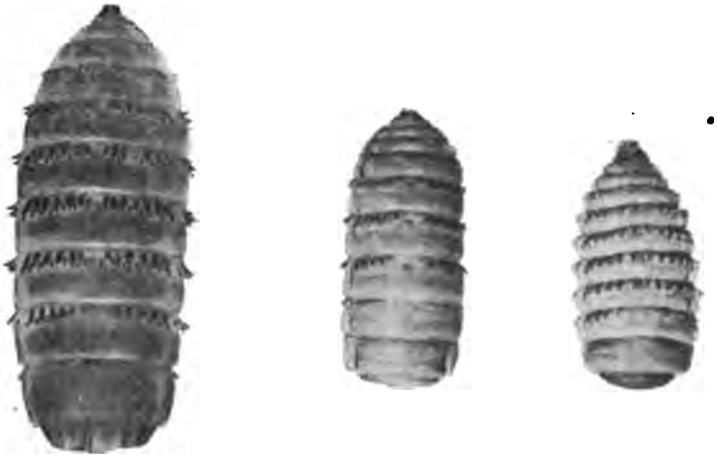

Fig 5. — Larves de *Gastrophilus* vues par la face dorsale, × 2, 5. — A gauche, larve de *G. intestinalis* (Degeer) Leach ; au milieu, larve de *G. hæmorrhoïdalis* (L.) Leach ; à droite, larve de *G. nasalis* (L.) Schiner.

Longueur du corps, 13 à 16mm ; largeur au 6e anneau, 6mm.

La longueur de l'anneau céphalique est égale à son diamètre transversal à la base.

Petites épines du 1er anneau disposées sur quatre rangées environ à la face inférieure et sur deux ou trois seulement à la face supérieure.

Il existe une double rangée d'épines alternes au bord antérieur des anneaux, du 2e au 9e inclusivement à la face ventrale et du 2e au 8e à la face dorsale. Les épines du second rang paraissent à peine plus petites que celles du premier. Comme chez la larve précédente, les couronnes sont interrompues sur la ligne médiane dorsale ; l'interruption, déjà large au 7e anneau, devient encore plus large au 8e. Les épines font complètement défaut sur toute la partie dorsale du 9e anneau. Les 10e et 11e anneaux sont entièrement dépourvus d'épines (1).

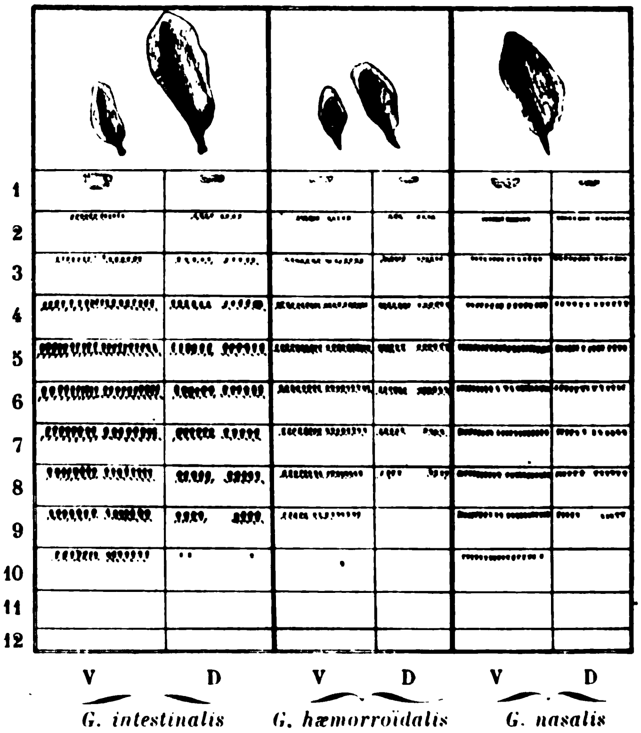

Fig. 6. — Tableau montrant la forme et la disposition des épines des larves de *G. intestinalis* (Degeer) Leach, *G. hæmorrhoïdalis* (L.) Leach et *G. nasalis* (L.) Schiner. Les chiffres placés à gauche des colonnes horizontales indiquent les numéros d'ordre des anneaux ; le n° 12 désigne les lèvres situées à la partie postérieure du 11e anneau. — La colonne V désigne la face ventrale et la colonne D, la face dorsale. — Gross. des épines = 17 f.

Les épines (fig. 6) sont coniques, d'un brun clair à la base, plus foncées à la pointe que chez le *G. intestinalis* ; l'extrémité libre n'est pas terminée par un renflement fendu transversalement.

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(1) Brauer (6) signale des épines au bord antérieur et ventral du 10e anneau, plus petites qu'aux anneaux précédents. Sur les 5 larves que j'ai examinées, une seule présentait 3 épines à peine visibles à cet anneau, les 4 autres en étaient totalement privées. Il existe peut-être plusieurs variétés de la même espèce.

3° **Larve de *G. nasalis*** (L.) Schiner (fig. 4, 5 et 6). — Forme plus ovoïde que dans les deux espèces précédentes. La couleur, d'abord blanche, avec extrémité postérieure rouge, devient d'un blanc jaune cire lorsque les larves ont achevé leur croissance.

Longueur du corps, 13 à 15^{mm}; largeur au septième anneau, 6^{mm}.

La longueur de l'anneau céphalique est égale à son diamètre transversal à la base.

Epines du premier anneau très petites, disposées sur quatre rangées environ à la face inférieure et sur deux seulement à la face supérieure. C'est à peu près la même disposition que chez la larve de *G. hæmorrhoïdalis*.

Les anneaux porteurs d'épines sont les mêmes que chez le *G. intestinalis*, mais au lieu de deux rangées on n'en trouve plus qu'une au bord antérieur de chacun d'eux. Cette unique rangée correspond au premier rang des deux larves précédentes. Elles existent, par conséquent, à partir du deuxième jusqu'au dixième anneau inclus à la face ventrale et jusqu'au neuvième seulement à la face dorsale.

Sur le milieu du neuvième, dorsalement, la couronne présente une interruption assez considérable. Le onzième anneau seul est entièrement nu.

Les tubercules épineux (fig. 6) sont blancs à la base, brun foncé à l'extrémité libre. Celle-ci est terminée en pointe, sans renflement.

J'ai résumé dans le tableau suivant les caractères qui permettent d'arriver rapidement à la détermination des trois espèces de larves étudiées ci-dessus :

Epines (sauf celles du 1er anneau) disposées :

- *Sur 2 rangées* alternes, les épines de la 1re rangée étant plus développées que celles de la seconde.
  Face ventrale du 10e anneau et face dorsale du 9e :
  - Munies de nombreuses épines, comme aux anneaux précédents.
    11e anneau seul, complètement dépourvu d'épines.
    L'extrémité libre des épines est terminée par un renflement sphérique divisé en deux par une fente transversale. . *G. intestinalis* (Degeer) Leach.
  - Nues ou présentant quelques rares épines très petites.
    10e et 11e anneaux, complètement dépourvus d'épines.
    L'extrémité libre des épines est pointue, sans renflement. *G. hæmorrhoidalis* (L.) Leach.
- *Sur une rangée :*
  11e anneau seul, complètement privé d'épines.
  Extrémité libre des épines pointue, sans renflement .. *G. nasalis* (L.) Schiner.

LÉSIONS CAUSÉES PAR LES LARVES DE *Gastrophilus intestinalis* (DEGEER) LEACH.

Lorsqu'on ouvre l'estomac d'un Cheval que l'on vient de sacrifier, il est bien rare, à défaut de larves, de ne pas y trouver des traces plus ou moins nombreuses de leur implantation dans la muqueuse. Presque tous ceux que j'ai eu l'occasion d'observer renfermaient des larves dont le nombre variait entre 2 et 358, mélangées à des lésions en plus ou moins grande quantité ; plus il y avait de lésions, moins nombreux étaient les parasites, et inversement. Il est fréquent d'en rencontrer une centaine à la fois, mais ce nombre est souvent dépassé : Vallisnieri (**43**) en a compté 600, Numan (**28**) 1013 et même davantage, dans le même estomac.

En France, les larves les plus communes sont celles de *G. intestinalis* (Degeer) Leach, ensuite viennent celles de *G. hæmorrhoïdalis* (L.) Leach et de *G. nasalis* (L.) Schiner. J'en ai recueilli 1120 dans 12 estomacs de Chevaux, à Rennes, dans le courant de l'été der-

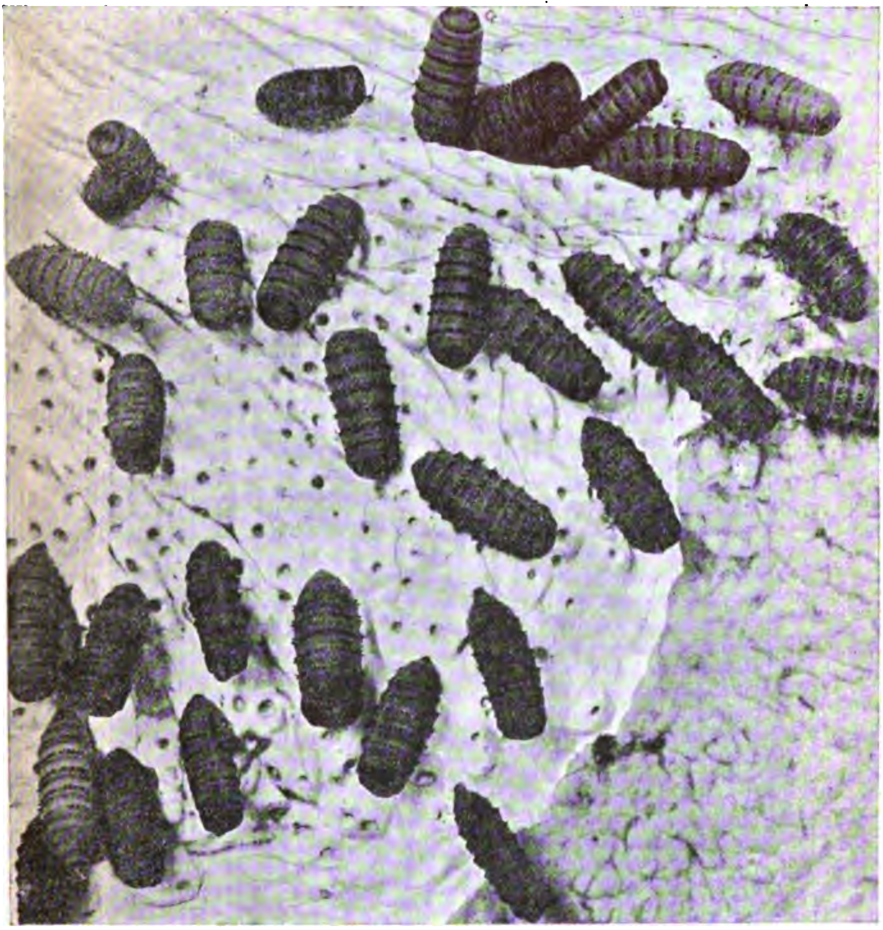

Fig. 7. — Portion de la muqueuse gastrique du Cheval envahie par les larves de *Gastrophilus intestinalis* (Degeer) Leach. Grandeur naturelle. On aperçoit nettement la ligne de séparation des deux sacs. Des larves sont implantées sur la muqueuse du sac gauche, mélangées à des alvéoles vides.

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nier : une seule appartenait à la seconde espèce, toutes les autres étaient des larves de *G. intestinalis*.

Leur endroit de prédilection est le sac gauche de l'estomac ; elles sont groupées sur cette portion de la muqueuse gastrique au voisinage de la crête qui la sépare de celle du sac droit, la tête enfoncée dans des alvéoles (fig. 7). Il est exceptionnel, dit Railliet (**33**), de les voir installées sur la muqueuse veloutée du sac droit ; lorsqu'on les trouve en cet endroit, le plus souvent ce sont des

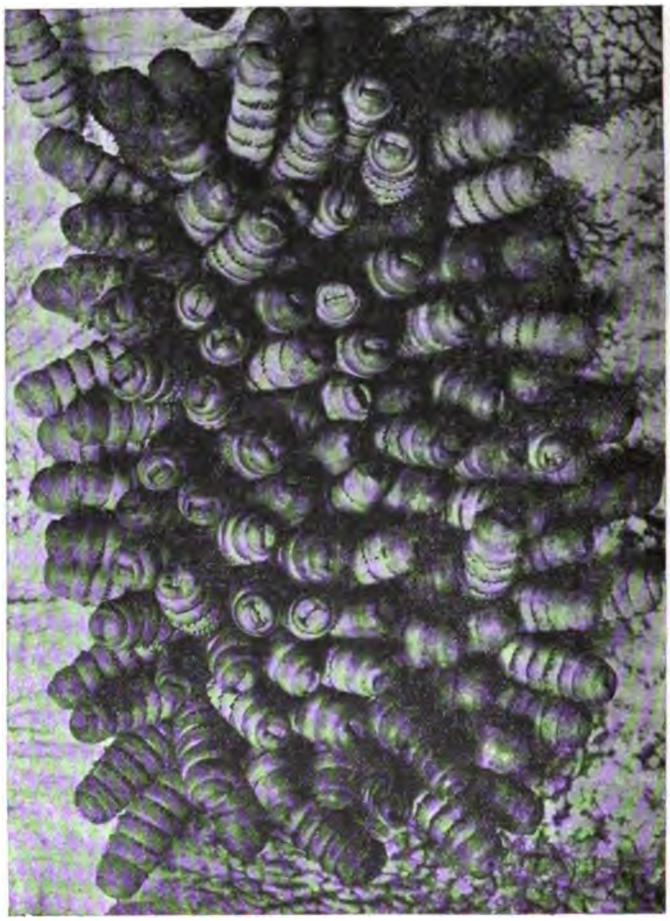

Fig. 8. — Groupe de larves de *Gastrophilus intestinalis* (Degeer) Leach fixées sur la muqueuse du sac droit de l'estomac du Cheval. Grandeur naturelle.

larves qui se sont déplacées après la mort de l'hôte. Mais alors elles ne sont pas fixées dans des alvéoles. Puisque les exemples en sont très rares, je pense qu'il n'est pas sans intérêt de signaler les deux cas suivants.

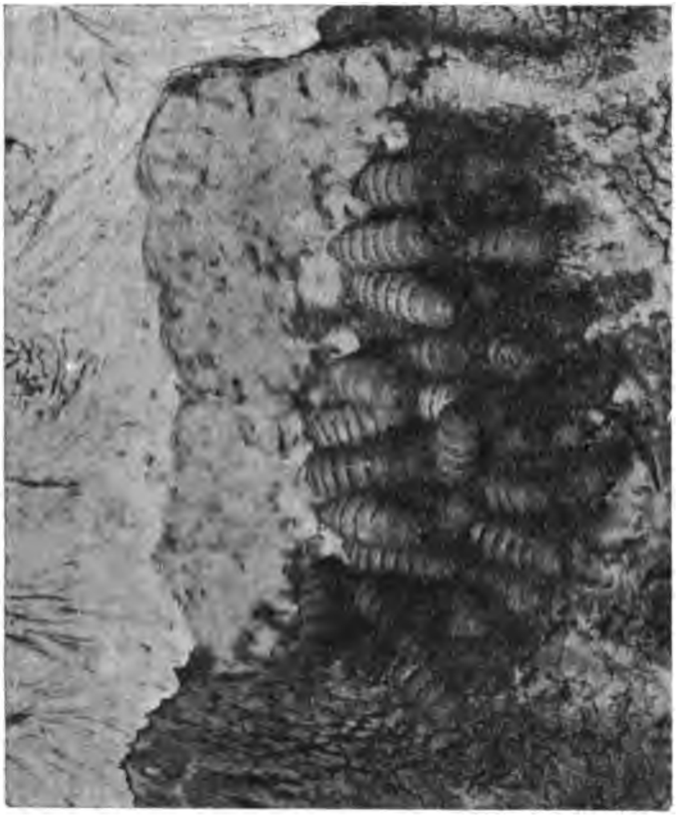

Fig. 9. — Même groupe de larves que dans la figure 8. Les larves les plus voisines de la limite de séparation des deux sacs ont été enlevées pour montrer qu'elles étaient fixées dans des alvéoles de la muqueuse gastropylorique. Grandeur naturelle.

Dans le premier (fig. 8), les larves, au nombre de 119, sont groupées, serrées les unes contre les autres, sur un espace à peu près rectangulaire mesurant 11 centimètres de long sur 8 centimètres de large. L'ensemble forme une grappe à cheval sur la ligne festonnée qui sépare les deux sacs, mais tandis qu'elle empiète d'un centimètre à peine sur la muqueuse gauche, elle envahit la partie droite sur une longueur de 7 centimètres. Je me suis assuré qu'à ce niveau la crête intermédiaire aux deux sacs ne faisait pas un détour à droite pour englober la masse des parasites. J'ai détaché de la paroi les larves qui cachaient cette ligne (fig. 9) et me suis convaincu que celle-ci ne s'écartait pas sensiblement de sa direction normale. A la place de chaque larve existait un alvéole caractéristique et suffisamment profond pour attester qu'il était de date ancienne.

Dans le second cas, les larves étaient au nombre de 28 et appartenaient toutes à l'espèce *Gastrophilus intestinalis* (Degeer) Leach, comme ci-dessus. Elles occupaient sur la muqueuse gastropylorique un espace de 40 centimètres carrés environ ; mais ici elles étaient suffisamment espacées pour que l'on pût, à première vue, s'assurer qu'elles étaient bien fixées sur cette portion de la muqueuse gastrique.

Les deux pièces dont il vient d'être question, placées dans le formol, sont conservées dans les collections de la Faculté des Sciences de Rennes.

ANATOMIE PATHOLOGIQUE DES LÉSIONS

Je laisse volontairement de côté les lésions rares, telles que les perforations de l'estomac ; je m'occuperai seulement de celles que l'on observe habituellement.

Les nombreux auteurs qui se sont occupés des larves gastricoles ont tous vu et décrit, d'une façon sommaire, les ulcérations qui garnissent la surface de la muqueuse stomacale des Equidés envahis par les Œstres. A cause sans doute du peu d'importance qu'elles paraissent avoir, ils se sont bornés à décrire la forme et les dimensions des lésions vues à l'œil nu. Aucun d'eux n'a eu la curiosité de les examiner au point de vue histologique.

Cependant Wedl (**44**), à propos de la cicatrisation des plaies laissées par le *Gastrophilus intestinalis* (Degeer) Leach, donne les dimensions de l'alvéole et s'exprime ainsi : « dans cette cavité se dépose peu à peu du tissu épithélial qui est soulevé par l'obturation progressive de la cavité par du tissu conjonctif de nouvelle formation. Ce tissu épithélial, formé de plusieurs couches, se présente sous la forme d'une plaque de favus. La coloration jaune-sale de la cicatrice est due à la nécrose de l'épithélium et aux nombreux bacilles en bâtonnets qui s'y trouvent. Finalement il ne reste plus qu'une dépression scutelliforme. »

Ces quelques lignes constituant à ma connaissance l'unique document sur la question, j'ai recherché quelles étaient les modifications histologiques apportées dans la muqueuse gastrique par les parasites dont elle est si souvent infestée.

TECHNIQUE. — La petite portion d'estomac que l'on désire étudier doit être prélevée le plus tôt possible après la mort du Cheval, afin d'éviter les phénomènes d'autodigestion qui s'effectuent dans le viscère après qu'il a été séparé de l'animal. Cette précaution n'a toutefois qu'une importance relative lorsqu'il s'agit, comme dans le cas présent, du sac gauche. Ce dernier est, en effet, tapissé d'un épithélium pavimenteux stratifié du type épidermique très résistant, sur lequel le suc gastrique a peu d'action.

Dans les conditions où je me suis trouvé, j'étais en possession du viscère une heure environ après que le Cheval eût cessé de vivre.

On découpe le fragment à examiner et on le plonge aussitôt dans un liquide fixateur. Celui qui m'a donné les meilleurs résultats est le formol picrique ou *liquide de Bouin.*

Après fixation, on traite la pièce, selon la méthode habituelle, successivement par l'alcool à 70°, l'alcool à 90°, l'alcool absolu, un mélange à parties égales d'alcool absolu et de xylol, le xylol pur, un mélange à parties égales de paraffine et de xylol, puis on inclut dans la paraffine pure.

Les coupes, après double coloration à l'hémalun et l'éosine sont déshydratées, éclaircies à l'essence de girofle et montées dans le baume.

Avant de commencer l'étude microscopique des lésions, je pense qu'il n'est pas inutile d'indiquer, en quelques mots, la structure normale de l'estomac du Cheval.

ANATOMIE NORMALE DE L'ESTOMAC DU CHEVAL. — Si l'on consulte le traité d'anatomie comparée de Chauveau et Arloing (**9**) on y trouve que ce viscère est un sac membraneux, d'une capacité de 14 à 15 litres, étranglé dans sa partie moyenne et dans lequel on distingue : 1° deux faces, l'une *antérieure*, l'autre *postérieure ;*

2° une *grande courbure* ou *courbure convexe ;* 3° une *petite courbure* ou *courbure concave ;* 4° une *extrémité gauche* ou *cul-de-sac gauche*, renflée en forme de grosse tubérosité ; 5° une *extrémité droite* ou *cul-de-sac droit*, moins volumineuse que la précédente, recourbée en haut et continue avec le duodénum.

Quand on examine l'estomac ouvert, on est frappé du contraste qui existe entre sa partie gauche et sa partie droite ; la ligne de démarcation est représentée par une crête saillante, sinueuse, extrêmement nette, faisant tout le tour de l'organe. C'est à cette crête que correspond, à l'extérieur, la dépression circulaire qui sépare les deux sacs. Dans la partie gauche, appelée *sac gauche*, la muqueuse est blanche, sèche, résistante et recouverte d'une épaisse couche d'épithélium. Elle est identique à celle de l'œsophage ; cette portion de l'estomac est d'ailleurs considérée comme un évasement du conduit œsophagien. A droite, la muqueuse devient épaisse, ridée, spongieuse, très folliculeuse, prend une teinte rouge brunâtre, rendue marbrée par des taches beaucoup plus foncées, perd de sa consistance et ne paraît plus revêtue que d'une légère pellicule épithéliale. C'est le *sac droit*, le véritable estomac des Solipèdes où s'élabore le suc gastrique. Le *cardia* se trouve à l'entrée du sac gauche, le *pylore* termine l'extrémité du sac droit.

Parois. — Les parois sont formées de trois tuniques : 1° une membrane externe, *séreuse*, dependance du péritoine ; 2° une membrane moyenne, *musculeuse*, constituant le *muscle moteur* de l'estomac. Au niveau du sac gauche, ce muscle est formé de 3 plans superposés de fibres musculaires : un plan moyen, un plan superficiel et un plan profond. Les fibres, circulaires dans le plan moyen, sont disposées en anses dans les deux autres de telle sorte que les fibres d'un plan croisent la direction des fibres de l'autre ; 3° une membrane interne, *muqueuse*, séparée de la précédente par du tissu conjonctif lâche qui lui permet de glisser facilement sur la tunique musculeuse.

Muqueuse. — N'ayant trouvé que des renseignements épars et incomplets sur la structure de la muqueuse du sac gauche, la description suivante se rapporte à mes observations personnelles.

Lorsqu'on examine une coupe perpendiculaire à la surface de cette membrane (fig. 10), on s'imagine avoir devant les yeux une section de la peau d'un Mammifère. L'étonnement sera de peu de durée si l'on songe qu'elle n'est qu'un prolongement de la muqueuse de l'œsophage dont la structure est analogue à celle de la peau. Comme cette dernière elle est dermopapillaire, avec épithélium du type malpighien, mais elle possède en plus un muscle qui lui est propre, la musculaire muqueuse (*muscularis mucosæ*), qui n'existe pas dans la peau. Elle comprend, en allant de l'intérieur de la cavité vers l'extérieur :

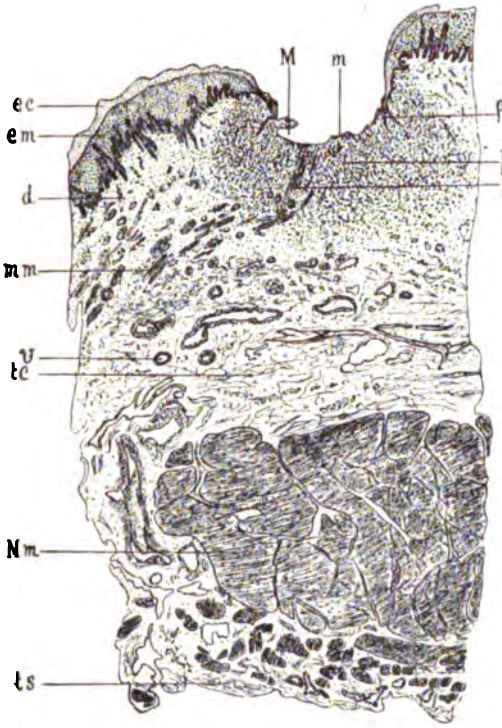

Fig. 10. — Coupe perpendiculaire à la paroi du sac gauche de l'estomac du Cheval, passant par le milieu d'un alvéole creusé par la larve de *Gastrophilus intestinalis* (Degeer) Leach. × 9. — *d*, derme de la muqueuse ; *ec*, couche superficielle de l'épithélium ; *em*, corps muqueux de Malpighi ; *f*, fente dans le derme ; *M*, diverticule où était logé le crochet mandibulaire ; *m*, dépression qui recevait les deux mâchoires ; *Mm*, muscle moteur de l'estomac ; *mm*, musculaire muqueuse ; *p*, partie du derme envahie par les leucocytes ; *tc*, tissu conjonctif sous-muqueux ; *ts*, tissu conjonctif sous-séreux ; *v*, vaisseau de la sous-muqueuse ; *z*, musculaire muqueuse relevée vers le fond de l'alvéole.

1° *Epithélium pavimenteux stratifié* du type des muqueuses dans lequel on distingue : 1° une couche superficielle dont toutes les cellules, jusqu'à la surface, possèdent des noyaux (*ec*) ; 2° un réseau muqueux de Malphigi (*em*) ; 3° une couche génératrice ;

2° *Derme* (*d*) pourvu de papilles du type adélomorphe, c'est-à-dire qu'elles sont noyées dans l'épithélium sans faire saillie à la surface libre de la muqueuse ;

3° *Musculaire muqueuse* (*mm*).

Selon Renaut (35), les glandes œsophagiennes, situées chez les autres Mammifères au-dessous de la musculaire muqueuse, font défaut chez le Cheval. Je me suis assuré, ce qui était facile à prévoir, qu'elles n'existent pas davantage au niveau du sac gauche.

La muqueuse du sac droit comporte :

1° Un *épithélium cylindrique* dont toutes les cellules sont mucipares et du type caliciforme ;

2° Une *couche glanduleuse* ;

3° Une *musculaire muqueuse*, qui envoie des prolongements entre les glandes de la couche précédente et qui est beaucoup plus épaisse que celle du sac gauche.

Lésion examinée au microscope. — Je suppose une coupe passant par le centre d'un alvéole (fig. 10). La lésion est creusée uniquement aux dépens de la muqueuse ; le fond de la cavité n'atteint même pas le tissu conjonctif sous-muqueux. Quant au muscle moteur je n'y ai jamais trouvé, à ce niveau, la dépression signalée par quelques auteurs ; je suis toutefois disposé à croire qu'elle existe, lorsque la larve s'est enfoncée au delà de son premier anneau.

La lésion a la forme d'une petite cupule limitée à la surface par un bourrelet circulaire. Son diamètre, qui est de 2^{mm} à l'entrée, diminue ensuite progressivement jusqu'au fond de la cavité ; elle a 1^{mm} 5 de profondeur. Mais ces dimensions peuvent atteindre 4^{mm} en largeur sur 3^{mm} en profondeur. On peut même voir deux larves fixées dans le même alvéole ; celui-ci présente alors nécessairement une largeur plus grande.

La partie centrale du fond de la cavité offre une petite dépression (fig. 10, *m*) à bords surélevés, occupée par les deux mâchoires. Peu visible dans la fig. 8, où la coupe n'est pas médiane, on la distingue mieux dans la fig. 7. Les parties latérales forment deux diverticules pour loger les deux crochets mandibulaires (fig. 10, *M*).

Les parois de la cupule sont constituées en partie aux dépens de l'épithélium, en partie aux dépens du derme de la muqueuse. La couche superficielle épithéliale et le corps muqueux de Malpighi taillés en biseau sont incomplètement détruits au niveau de la lésion. On dirait qu'en s'enfonçant dans la muqueuse, la larve les a entraînés à sa suite. L'épithélium a totalement disparu dans la partie profonde de la cavité dont le fond est formé par le derme de la muqueuse.

Au voisinage de la lésion, l'épithélium a conservé, sauf l'épaisseur, les caractères qu'il possède dans les endroits sains de la membrane : les cellules de la couche superficielle sont toutes munies de noyaux jusqu'à la surface ; le corps muqueux de Malpighi ne paraît avoir subi aucune modification notable.

C'est dans le derme qu'il faut chercher les dégâts occasionnés par la larve : on y remarque une abondante infiltration leucocytaire (fig. 10, *p*) ayant la forme d'un cercle dont le centre occuperait le milieu du fond de l'alvéole et qui serait interrompu du côté libre de la muqueuse par l'encoche due à la larve. Le nombre des globules blancs va en diminuant dans tous les sens à mesure qu'on s'éloigne du centre de la lésion, de telle sorte qu'au niveau de la sous-muqueuse il n'y a plus que quelques rares cellules embryonnaires. La tunique musculaire est indemne. La musculaire muqueuse, si nette dans les régions saines, est dissociée et se relève vers le fond de la cupule (fig. 10, *z*) ; il semble qu'elle ait été aspirée par les mouvements de succion du parasite ; visible dans la partie gauche de la figure elle a complètement disparu à droite.

Les éléments de la zone malade sont formés des leucocytes qui ont traversé par diapédèse les parois des vaisseaux des environs, et des cellules fixes du tissu conjonctif dermique revenues à l'état embryonnaire. Je n'ai jamais vu la bouche de la larve appliquée sur un vaisseau.

Les dégâts se résument par conséquent en une destruction d'une partie de la muqueuse et en une petite inflammation localisée se traduisant par une agglomération dans le derme de nombreux globules blancs autour du point d'implantation de la larve. C'est une réaction inflammatoire banale analogue à celle qui se produit autour d'un corps étranger quelconque.

On comprend, dès lors pourquoi les Chevaux ne paraissent pas souffrir de la présence de ces parasites dans un organe aussi important que l'estomac. On s'expliquera encore mieux ce fait si l'on songe que les larves sont en général fixées dans la partie de ce viscère où les glandes sont absentes. Elles n'occasionnent de cette façon aucun trouble dans la sécrétion du suc gastrique et par suite n'apportent pas d'entrave appréciable dans les fonctions digestives.

Mode d'implantation de la larve. — Pour savoir comment la larve est fixée à la muqueuse, il est nécessaire que la section soit perpendiculaire à la paroi de l'estomac et coupe en même temps le premier anneau de l'insecte suivant un plan qui passe à la fois par les deux crochets mandibulaires.

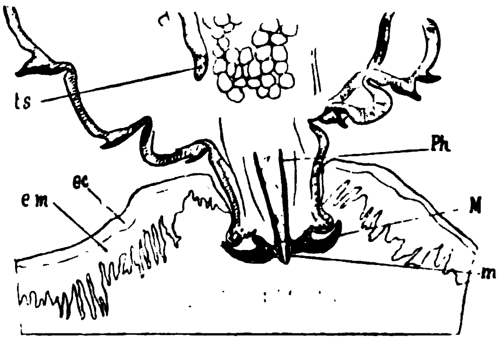

Fig. 11. — Partie antérieure d'une larve de *Gastrophilus intestinalis* implantée dans la muqueuse gastrique gauche du Cheval. × 11. — *ec*, couche superficielle de l'épithélium ; *em*, corps muqueux de Malpighi ; *M*, Crochet mandibulaire de la larve ; *m*, mâchoire ; *Ph*, pharynx ; *ts*, organe spongieux terminant une des grosses trachées longitudinales de la larve.

Il est assez difficile d'obtenir une telle préparation, mais à l'aide de coupes en série convenablement choisies on peut déterminer d'une façon suffisamment exacte la manière dont le parasite occupe son alvéole. C'est ainsi que je suis parvenu à représenter (fig. 11), sans être trop schématique, le mode de fixation de la larve de *Gastrophilus intestinalis* (Degeer) Leach.

L'anneau céphalique remplit à peu près complètement la cavité. L'intervalle compris entre les parois de l'alvéole et l'animal, insignifiant sur les côtés, est presque nul dans le fond ; la larve y applique exactement l'extrémité antérieure de la tête. Les crochets mandibulaires et les mâchoires se moulent dans les dépressions signalées lors de la description de la cavité alvéolaire.

La largeur et la profondeur de la cupule sont en rapport avec les dimensions de l'anneau céphalique de la larve. Celle-ci semble astreinte à arrêter son action destructive lorsque son premier anneau est complètement engagé dans la muqueuse; par suite de l'augmentation rapide de largeur des anneaux suivants, l'Insecte ne peut plus avancer dans les tissus de l'hôte. Parfois cependant il réussit à enfoncer son 2^e anneau; l'alvéole est dans ce cas plus considérable. C'est qu'alors, craignant sans doute d'être entraîné hors de la muqueuse par les matières alimentaires solides qui balayent la face interne de l'estomac, l'animal opère des tractions plus fortes et arrive ainsi à faire pénétrer une plus grande partie de son corps dans la paroi gastrique.

EXPLICATION DU SÉJOUR DES LARVES DANS L'ESTOMAC, TIRÉE DE L'ÉTUDE DES LÉSIONS.

Après un examen attentif des désordres produits sur la muqueuse stomacale et de la manière dont la larve y est implantée, j'ai cherché à donner une interprétation à un certain nombre de questions qui se présentent naturellement à l'esprit.

1. — *Pourquoi les observe-t-on exclusivement dans le sac gauche?*

Il est probable que les larves arrivant dans l'estomac se fixent n'importe où mais que, seules, celles de gauche trouvent dans l'épithélium pavimenteux stratifié de cette portion de l'organe une base d'implantation suffisamment résistante pour leur permettre de s'y fixer solidement.

Celles qui s'implantent dans la membrane molle et friable du sac droit, à épithélium formé d'une seule assise de cellules sans cesse renouvelée, sont délogées à la première occasion. Ce n'est qu'exceptionnellement qu'elles arrivent à s'y maintenir.

2. — *Pourquoi sont-elles groupées dans le voisinage de la crête qui sépare les deux sacs?*

Cette question restera très embarrassante tant qu'on ne saura pas exactement de quoi se nourrissent ces êtres singuliers. Peut-être les substances élaborées dans le sac droit ont-elles une action efficace sur le développement des larves; celles-ci se placeraient alors le plus près possible de ces substances, tout en se fixant en dehors de la muqueuse gastropylorique où il leur est impossible de se maintenir.

3. — *De quoi se nourrissent-elles?*

Bracy Clark (**5**) désignait les larves de l'estomac sous le nom de gastricoles chylivores, croyant qu'elles se nourrissaient du produit de la digestion stomacale.

Puisqu'on les a rencontrées dans l'œsophage et le pharynx, il est bien évident qu'elles ne vivent pas de cette matière, car il n'y en a pas dans ces régions. Si l'on admet que d'autres animaux de la même famille, l'Œstre du Mouton (*Œstrus ovis* L.) et l'Hypoderme du Bœuf (*Hypoderma bovis* Degeer) se nourrissent, le premier du mucus secrété par la muqueuse du nez et des sinus frontaux du Mouton, le second, du pus de l'abcès qu'il a créé en s'introduisant sous la peau du Bœuf, il sera permis de supposer que les Œstres de l'estomac trouvent leur aliment dans les produits inflammatoires de la muqueuse gastrique.

Le mode d'implantation de la larve prouve en faveur de cette hypothèse. Le parasite est en effet si intimement appliqué au fond de l'alvéole par son anneau céphalique que les liquides de l'estomac ne peuvent arriver à sa bouche ; c'est par conséquent dans la paroi même qu'il est obligé de chercher sa nourriture.

Il m'a semblé reconnaître entre les deux lames chitineuses du pharynx (fig. 11, *Ph*) un organe dont le rôle serait de servir de piston lorsque la larve exerce des mouvements de succion.

Malgré mes recherches je n'ai pu découvrir de globules blancs ou rouges dans le pharynx, ni même entre les mâchoires. Si ces éléments ne servent pas à la nutrition, il devient alors très probable que celle-ci s'effectue aux dépens de la partie liquide de l'inflammation, c'est-à-dire du plasma conjonctif exsudé, comme cela arrive partout où il y a irritation d'un tissu quelconque.

VITALITÉ DES LARVES ADULTES DE *Gastrophilus intestinalis* (DEGEER) LEACH.

Dans le but de débarrasser les Chevaux des larves de lestomac, un grand nombre d'expérimentateurs les ont soumises à l'action de substances ayant un pouvoir toxique plus ou moins grand : tous ont été frappés du degré de résistance extrême que ces êtres opposent aux agents les plus violents. Numan (**28**) a prouvé qu'après un séjour de trois ou quatre heures dans des solutions d'arsenic, d'Assa fœtida, de Noix vomique, de strychine, de sulfate de cuivre, de sublimé, de Ciguë, d'Aconit, de Belladone, dans l'essence de térébenthine, elles continuaient à vivre un temps variant entre trois et dix jours. Mais placées dans le chlore, liquide ou gazeux, les vapeurs d'acide cyanhydrique, l'ammoniaque liquide, elles furent trouvées mortes au bout de quatre heures.

Bourgelat (8) les a vues demeurer vivantes au bout de quatorze jours dans l'huile et de six semaines dans l'eau, ce que Numan révoque en doute.

Je me suis livré à quelques expériences qui viennent confirmer celles qu'avaient faites auparavant ces deux auteurs.

1° Je plongeai, le 8 juin 1900, dans une solution aqueuse de formol à 3 °/₀ une portion d'estomac de Cheval recouverte de 358 larves. Le 13 juin, c'est-à-dire 5 jours après l'immersion, quelques larves restées accrochées à la muqueuse étaient encore vivantes.

2° Dans une solution saturée de sublimé corrosif dans l'eau distillée, j'ai vu des larves faire des mouvements une heure après que je les y avais mises ; au bout de deux heures elles avaient cessé de vivre.

3° Le 20 novembre 1900, on m'apportait un lambeau d'estomac couvert de larves provenant d'un Cheval tué le jour même. Ce lambeau plongé dans le formol à 3 °/₀ en était extrait six heures après et placé dans une chambre humide à la température du laboratoire.

Après m'être assuré, le 1er décembre, que les larves étaient encore vivantes, j'en détachai trois de la muqueuse et les installai dans un cristallisoir contenant juste assez d'eau pour les maintenir toujours humides, sans les recouvrir entièrement de ce liquide. Le 20 décembre, deux de ces larves étaient mortes et enlevées du vase ; la troisième était encore vivante le 13 janvier suivant. Cette larve a donc encore survécu 10 jours, attachée à l'estomac, dans l'air humide et 44 jours, libre, en tout 54 jours depuis la mort du Cheval, après un séjour de 6 heures dans le formol à 3 °/₀.

Le lambeau d'estomac, avec toutes les larves qui restaient, fut laissé dans la chambre humide jusqu'au 7 décembre. Les parasites étaient toujours vivants, mais le fragment de viscère, en pleine

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putréfaction, exhalait une odeur si repoussante que je plaçai, le jour même, le tout dans un cristallisoir plein d'eau froide; celle-ci fut dans la suite renouvelée plusieurs fois par jour. Le 7 janvier suivant, toutes les larves, sauf une, étaient mortes. L'unique larve qui avait manifesté quelques rares signes de vie était morte à son tour 2 jours plus tard. Cette larve, après un séjour de 6 heures dans le formol à 3 °/₀, avait donc survécu 48 jours après la mort du Cheval, dont 31 jours passés entièrement sous l'eau, constamment fixée à un lambeau d'estomac en putréfaction.

Larves de *Gastrophilus* chez l'Homme

On a donné le nom de *myiasis* ou de *myiase* au fait de la présence des larves de Diptères chez l'Homme, ainsi qu'aux accidents qu'elles déterminent. Ces Insectes appartiennent pour la plupart à la famlile des Œstrides ou à celles des Muscides.

Si l'on parcourt le *traité de zoologie médicale* de R. Blanchard (**2**), on remarque que presque toutes les larves d'Œstrides que l'on a rencontrées chez l'Homme se rapportent soit au genre Hypoderme pour l'Europe, soit au genre Dermatobie pour l'Amérique. On les trouve sous la peau où elles déterminent la production de tumeurs semblables à celles des larves de l'Hypoderme du Bœuf.

Les larves de Gastrophiles ont été bien rarement observées dans l'espèce humaine; cependant on a signalé en Russie (1) plusieurs cas de « *dermatomyiase* » provoqués par une petite larve de *Gastrophilus* qui se creuse des galeries rouges dans l'épiderme de l'Homme.

Dans sa thèse, Paul Lallier (**19**) cite, d'après Schoch (**39**), le cas d'une femme qui aurait eu dans l'intestin des larves de *Gastrophilus*; et il ajoute que si le cas est exact, c'est avec celui de Chichester le seul connu de la présence des larves d'Œstrides dans le canal digestif de l'Homme.

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(1) N. Kholodkovsky, Sur quelques rares parasites de l'Homme en Russie. *Archives de parasitologie*, I, p. 354, 1898.

CONCLUSIONS

1° Les œufs de *Gastrophilus intestinalis* (Degeer) Leach s'observent plus fréquemment et sont plus nombreux sur les Chevaux à robe de couleur que sur les Chevaux blancs.

Plus de trois mois après qu'ils ont été séparés du Cheval on peut encore y trouver des larves vivantes. Comme il est peu vraisemblable que les œufs aient besoin de tant de jours pour atteindre leur complète maturité, les jeunes larves doivent, par conséquent, posséder la propriété de rester en état de vie latente, jusqu'à ce que les conditions favorables à leur éclosion viennent à se produire.

Il est possible de voir l'éclosion s'effectuer assez tard (le 1er décembre) sur le Cheval même, si les conditions atmosphériques ne sont pas trop rigoureuses.

2° Le nombre des segments de la larve de *G. intestinalis* (Degeer) Leach au premier stade est le même que chez la larve adulte. Seulement, chez celle-ci, le 12e et dernier anneau est en grande partie caché, de sorte qu'elle paraît avoir un anneau de moins que la jeune larvule.

Les couronnes d'épines sont placées, comme chez la larve mûre, au bord antérieur des anneaux. A la partie ventrale de la couronne céphalique il existe, à la première rangée, huit épines beaucoup plus longues et plus mobiles que les autres.

Aux neuf anneaux qui suivent, chaque couronne est constituée par deux rangées d'épines alternes qui diffèrent de forme, selon l'anneau que l'on considère. Ce sont des tubercules chitineux coniques, fortement recourbés aux trois premiers anneaux, presque droits aux suivants. Chaque crochet de la première rangée est en outre recouvert incomplètement d'une autre pièce chitineuse.

Les dernières couronnes d'épines sont largement interrompues sur la ligne médiane dorsale, comme chez la larve adulte.

Les deux derniers anneaux seuls sont totalement dépourvus de tubercules épineux ; le dernier est terminé par deux tubes, plissés transversalement à la base, mais non rétractiles, et présentant, à leur extrémité libre, les deux orifices stigmatiques. A la surface du tégument, aussi bien du côté dorsal que du côté ventral, il existe de très petites épines disposées d'une façon régulière et symétrique par rapport au plan sagittal de l'animal.

Deux grosses trachées longitudinales courent le long du corps et vont se terminer postérieurement aux deux orifices stigmatiques.

Le squelette du pharynx est formé de deux lames chitineuses longitudinales et d'une pièce médiane en forme de stylet que l'on aperçoit entre les deux crochets mandibulaires.

Plongées dans l'eau et dans différents liquides, les jeunes larvules, fraîchement écloses, montrent qu'elles sont douées d'une grande résistance à l'asphyxie. Les liquides volatils seuls les tuent instantanément ou en quelques secondes.

3° J'ai observé la mue chez la larve de *Gastrophilus intestinalis* (Degeer) Leach à l'avant-dernier stade. A cette phase, la larve se compose du même nombre de segments, disposés de la même façon que chez la larve au dernier stade.

Elle possède une couronne d'épines de moins que cette dernière et chaque couronne se compose d'une triple ou d'une quadruple rangée d'épines très petites et alternes d'un rang à l'autre.

Les arcs des plaques stigmatiques, peu courbés, sont au nombre de deux de chaque côté.

Si l'on remarque qu'il en existe trois chez l'adulte, alors qu'il n'y en a pas du tout chez la larve au premier stade, il est vraisemblable d'admettre une quatrième forme larvaire qui aurait sa place entre la première et l'avant-dernière phase. Le développement de la larve comprendrait ainsi quatre stades séparés par trois mues.

4° Les épines de la larve de *Gastrophilus intestinalis* (Degeer) Leach au dernier stade présentent à leur extrémité libre un renfledivisé en deux transversalement. Ce caractère ne se retrouve pas chez les larves de *G. hæmorrhoïdalis* (L.) Leach ni de *G. nasalis* (L.) Schiner.

La disposition et la forme des épines permettent à elles seules de différencier rapidement ces trois espèces les unes des autres.

5° Les lésions provoquées par la larve de *Gastrophilus intestinalis* (Degeer) Leach sur l'estomac du Cheval se réduisent à une destruction d'une partie de la muqueuse ne dépassant pas le derme et en un afflux leucocytaire autour du point de fixation du parasite.

Les globules blancs proviennent à la fois de la diapédèse des vaisseaux de la muqueuse et de la sous-muqueuse, ainsi que des cellules fixes du tissu conjonctif dermique revenues à l'état embryonnaire. C'est une réaction inflammatoire banale analogue à celle qui se produit autour d'un corps étranger quelconque.

Du mode d'implantation de la larve on peut déduire que les liquides digestifs ne parviennent pas à sa bouche ; l'animal se nourrit fort probablement des produits de l'inflammation qu'il détermine.

6° D'après quelques observations que j'ai faites, j'ai constate que des larves adultes de *Gastrophilus intestinalis* (Degeer) Leach plongées dans le formol à 3 °/₀ vivaient encore cinq jours après leur immersion dans le liquide. Immergées dans le sublimé en solution saturée dans l'eau distillée, d'autres larves n'étaient pas mortes au bout d'une heure.

Une larve, après un séjour de six heures dans le formol à 3 °/₀, n'avait pas encore cessé de vivre au bout de cinquante-quatre jours.

Une autre larve, après être demeurée pendant six heures dans le formol à 3 °/₀, a survécu quarante-huit jours, quoique le lambeau d'estomac sur lequel elle était placée fût en putréfaction. Ceci démontre une fois de plus la grande vitalité de ces singuliers animaux.

7° Plusieurs cas de myase cutanée et de myase du tube digestif dus à des larves de Gastrophiles ont été signalés chez l'Homme.

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44. Wedl, voir Scheiber, *loc cit.*

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an, J. de
10. Het eerste tweejaarlijksch
Congres van the Far-Eastern
Association of Tropical
Medicine, gehouden te
Manila van 5 - 14 Maart 1910.

Het eerste tweejaarlijksch Congres van The Far-Eastern Association of Tropical Medicine, gehouden te Manila van 5—14 Maart 1910.

DOOR

Dr. J. de HAAN.

*

Overgedrukt uit het
Geneeskundig Tijdschrift voor Nederlandsch-Indië,
Deel L, afl. 3.

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BATAVIA
JAVASCHE BOEKHANDEL & DRUKKERIJ.
1910.

Het eerste tweejaarlijksch Congres van The Far-Eastern Association of Tropical Medicine, gehouden te Manila van 5—14 Maart 1910,

DOOR

Dr. J. de HAAN.

Op den 27en Februari 1908 werd in de Philippine Medical School te Manila een vergadering gehouden door afgevaardigden van de Philippijnen (Manila), Ceylon, Hongkong, The Federated Malay States, Singapore, The Straits Settlements, Japan en China, ten einde de wenschelijkheid te overwegen een vereeniging te stichten van hen, die op het gebied der tropische geneeskunde in het verre Oosten werkzaam zijn. Reeds 2 dagen later was het besluit tot het oprichten eener zoodanige vereeniging genomen, en een concept der statuten gereed. Als naam voor de vereeniging werd gekozen dien van FAR-EASTERN ASSOCIATION OF TROPICAL MEDICINE. Haar doel werd omschreven in artikel 2 der Statuten als volgt: „The object of this Association shall be to promote the science and art of tropical medicine in the Far East. „For this purpose the Association shall endeavour to unite „into one compact organisation the medical profession „of the far east, for the growth and diffusion of medical „knowledge; to promote friendly international intercourse „among physicians; to elevate the standard of medical „education; to enlighten and direct public opinion in regard „to the problems of hygiene; to form habits which may „conduce to the prevention of disease among the native

„populations; and to present to the world the results of „such scientific investigations".

Omtrent het lidmaatschap bepaalt artikel 3: „The mem„bership of this Association shall consist of the members „of all duly constituted regularly organised medical societies „within the field of this organisation, and all medical offi„cials in the civil, military, naval or other organised services „of Governements within these territories". In artikel 5 „werd o.a. bepaald: The Association shall hold a biennial „session at a time and place to be fixed by the Council" en in artikel 7, dat de jaarlijksche onkosten aan het lidmaatschap verbonden zouden bedragen $ 5 Philippine currency = ± *f* 6.—.

De eerste tweejaarlijksche bijeenkomst werd bepaald op 5-14 Maart te Manila en het volgende programma vastgesteld:

AT MANILA.

Saturday afternoon, March	5	Opening session.
Sunday		No session.
Monday, March	7	Protozoology, Helminthology.
Tuesday, „	8	Cholera, Plague, and Leprosy.
Wednesday, March	9	Surgery and Obstetrics, Diseases of children.
Thursday, „	10	Fevers in the tropics, including Malaria, Typhoid etc.
Friday, „	11	Dysenteries; Beri-Beri.
Saturday, „	12	Tuberculosis.
Saturday night		En route to Baguio.

AT BAGUIO.

Sunday		No session.
Monday, March	14	Climate, Hygiene and Sanitation; Business session.

Return to Manila Monday-night.

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Ofschoon het schijnt, dat oorspronkelijk bij de Regeering het plan bestond om slechts één afgevaardigde uit onze koloniën aan deze bijeenkomst te doen deelnemen en schrijver dezes daarvoor werd aangewezen, werden later ook nog de officier van gezondheid der 1e klasse van de landmacht H. M. NEEB en die van Hr. Ms. Zeemacht Dr. G. J. VAN DER SANDE van een gelijkluidende opdracht voorzien, zeker een blijk van groote belangstelling onzer Regeering in den wetenschappelijken arbeid, die allerwegen door de geneeskundigen in de tropen met grooten (ook soms te haastigen?) ijver wordt verricht, waardoor tevens een niet genoeg te waardeeren gelegenheid gegeven werd tot vergelijking op verschillend gebied van toestanden hier te lande met die op de Philippijnen. En al moge daarbij vooral niet uit het oog worden verloren, dat „omnis comparatio claudicat," omdat noch de menschen, noch de omstandigheden overal dezelfde zijn, gewapend met een goede dosis kritiek, zou zeker uit het geen ons oog aldaar te zien en ons oor te hooren kreeg, wel een en ander geleerd kunnen worden, dat ook voor ons van belang kon zijn.

Met diepen weemoed vervult mij de herdenking aan den vrij plotselingen dood van Dr. VAN DER SANDE, waardoor ons een aangename reisgenoot, aan het congres zeer zeker een ijverig deelnemer ontviel, die door zijn standaardwerk over het ethnologisch gedeelte der Nieuw-Guinea-expeditie zijn naam als voortreffelijk geleerde voorgoed had gevestigd. Eere zij zijn nagedachtenis!

Op den 12en Februari aanvaardden wij de reis over Singapore en Hongkong en hadden daardoor een schoone gelegenheid om ook in deze groote middenpunten van verkeer een en ander op geneeskundig of hygiënisch gebied te zien. Een zonderlingen indruk maakt het op den reiziger, dat hij, alvorens te Hongkong voor Manila te worden ingescheept, verplicht is een formulier in te vullen met een groot aantal vragen, waaronder die of hij lezen en schrijven kan, anarchist is, 50 dollars bezit en wel eens in de gevangenis heeft gezeten, nog tot de bescheidenste behooren. Bovendien moet een bedrag gestort worden van 9,5 Hongkong dollar. Doch daarmede zijn de formaliteiten, die de poorten van het beloofde land voor ons zullen ontsluiten, nog niet ten einde, want alvorens te worden ontscheept, heeft op de reede van Manila aan boord een onderzoek plaats van de hut- en handbagage, en, na ontscheping, in het douane-kantoor van de grootere koffers, zóó nauwkeurig en gestreng, als geloof ik nergens ter wereld geschiedt. Een en ander ontlokte ons armen slachtoffers wel eens een minder vriendelijke verzuchting over de zoo hoog geroemde Amerikaansche vrijheid. Doch, aan alles komt een einde, dus ook aan het geharrewar met Amerikaansche douane-beambten en vrij warm en ontstemd hielden wij onzen intocht in Manila, met echt Amerikaansche bescheidenheid „the pearl of the Orient", ook wel „the dreamcity" genoemd. Hadden wij omtrent de hotels aldaar reeds een en ander vernomen, zoodat onze verwachtingen niet zeer hoog gespannen waren, de werkelijkheid bleef toch noch ver beneden deze. Ik wil er niet meer van zeggen dan dat zij zonder onderscheid zeer vuil zijn, het eten er......, nu ja, voor ons ongewoon is en de bediening alles te wenschen overlaat.

Aangezien wij reeds op den 28en Februari te Manila aankwamen en het Congres eerst den 5en Maart zou geopend worden, hadden wij een goede gelegenheid ons eenigszins nader omtrent deze stad en datgene wat de Amerikanen er, sedert zij zich van de Philippijnen meester maakten, tot stand brachten, op de hoogte te stellen. Dit werd ons zeer gemakkelijk gemaakt door de groote hulpvaardigheid van den Nederlandschen Consul te Manila, den Heer P. K. A. MEERKAMP VAN EMBDEN, die ons, met opoffering van veel voor hem kostbaren tijd, persoonlijk voorstelde aan den Vice-Gouverneur (de Gouverneur-Generaal

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was voor dienstaangelegenheden op reis), den Legercommandant, den Chef over den Geneeskundigen dienst en den Voorzitter van het Congres Dr. PAUL C. FREER. Bij al deze autoriteiten ontmoetten wij de meest voorkomende welwillenheid en hulpvaardigheid, zeer zeker niet het minst een gevolg van de groote ingenomenheid met ons volk en onze koloniën, zooals ons uit menig gesprek bleek, en de persoonlijke waardeering van onzen vriendelijken Consul.

Manila wordt wel eens de stad der contrasten genoemd. En terecht. Waar nog voor slechts ruim een tiental jaren deze stad sluimerde in den doezel van haar Oostersch bestaan, borrelt en bruist thans met geweldige kracht het nimmer rustende westersche leven: door dezelfde straten en langs dezelfde wegen, die gedurende eeuwen geen ander middel van vervoer kenden dan de rustig voortstappende karbouw of het voor een nog veel primitiever voertuig dan onze sado voortsukkelende inlandsche paardje, snort thans een stroom van electrische trams en auto's, als de dragers van het begrip, dat tijd geld en het leven slechts kort is.

De breede Pasig-river verdeelt de stad in een noordelijk en een zuidelijk gedeelte. Op den zuidelijken rivieroever liggen: de nog door haar wallen omgeven oude Spaansche stad, Manila intra muros, en de distrikten Ermita, Malate en Paco, terwijl op den noordelijken oever zich bevinden: de Escolta, het zeer levendige verkeerscentrum van Manila extra muros, en de distrikten Binondo, San Nicolas, Tondo, Santa Cruz, Quiapo en Sampolac. Noord- en Zuid-Manila zijn door een viertal bruggen met elkander verbonden, waarvan de Bridge of Spain de voornaamste is, wier krachtige, doch tevens schilderachtige bouw aan de typhonen van bijna drie eeuwen weerstand bood en een welsprekend getuigenis aflegt van de bouwkunst der Spanjaarden. In 1902 werd door Amerikaansche ingenieurs de Santa Cruz bridge gebouwd, om het verbazend groote verkeer, dat over de

Bridge of Spain plaats had, gedeeltelijk in een andere richting te leiden. De beide andere bruggen zijn: een particuliere hangbrug en de eerst sedert korten tijd voltooide Ayala Bridge. Verder wordt het groote aantal kanalen, dat Manila doorploegt, overspannen door een 50 tal smalle bruggen, die meest alle nog dagteekenen uit den Spaanschen tijd.

Onder deze kleinere bruggen in Manilla is er een, die geldt als een historisch gedenkteeken: de San Juan Bridge, geslagen aan het einde van de Santa Mesa-tramlijn over de San Juan River, den noordelijken arm van de Pasig-river. Over deze brug viel in den nacht van den 4en Februari 1899 het eerste schot, waarmede de strijd geopend werd, die aan den opstand der Philippinos tegen de Amerikanen (voor goed?) een einde zou maken. Schildwachten der beide partijen bezetten de tegenovergestelde einden der brug, en op genoemden avond vuurde een beschonken officier der opstandelingen op den Amerikaanschen schildwacht. De strijd, die met de onderwerping der Philippinos eindigde, nam hiermede een aanvang.

Aan de wallen van Manila intra muros, wier dikte afwisselt van 3 — 40 voet, is de geheele geschiedenis der Philippijnsche eilanden nauw verbonden. Bijna drie eeuwen waren noodig om ze tot stand te brengen (1591 — 1872), waarbij duizende menschenlevens en millioenen schats werden ten offer gebracht. Tot 1905 waren zij omgeven door een gracht van aanzienlijke breedte en diepte, doch de Amerikanen dempten deze gracht uit een hygiënisch oogpunt en gebruikten daarvoor het zand en slib, dat bij den aanleg der nieuwe havenwerken werd opgebaggerd. In deze wallen bevinden zich een aantal ruimten, die in vervlogen tijden gebruikt werden voor gevangenissen, en waarin door de Amerikanen tal van martelwerktuigen en menschenbeenderen werden gevonden, als stomme getuigen van naamloos menschelijk wee en vroegere Spaansche wreedheid.

Nu bekoren zij nog het oog door hunne fraaie tinten en de architektonische pracht der oorspronkelijk ten getale van zeven aanwezige poorten. Het Amerikaansche leven brak zich echter naar buiten baan door nog vier nieuwe openingen.

Op den noordwestelijken hoek van Manila intra muros staat het fort Santiago, een van de meest belangwekkende bouwwerken uit den Spaanschen tijd. In 1571 gebouwd als een verdedigingswerk tegen de krijgszuchtige Moros en andere wilde horden, bestond het slechts uit houten palissadeeringen, doch in 1590 werden deze, op last van den toenmaligen Gouverneur der eilanden SANTIAGO DE VERA, door steenen wallen vervangen en onder zijn opvolger DASMARINAS werd het fort voltooid, dat nu de krachtigste wijkplaats in de Philippijnen aanbood en waarbinnen het hoofdkwartier van het Spaansche Gouvernement gevestigd werd. En ofschoon ook nu nog het hoofdkwartier van de Philippine Division of the United States Army, heeft het geen krijgskundige beteekenis meer. Intra muros bevinden zich ook de bureaux van den Gouverneur-Generaal en der andere autoriteiten, in het fraaie Ayuntamiento, dat vroeger door den Spaanschen Gouverneur werd bewoond en het middenpunt vormde van het oude Spaansche regime, benevens het Bureau of Health, dat zelfbewust, met krachtigen arm de zegeningen der moderne gezondheidsleer over de Philippijnsche eilanden uitstrooit. De bewonderaar van fraaie kerken vindt intra muros ruimschoots gelegenheid om aan de aldaar aanwezige, van historische herinneringen en architektonisch schoon getuigende, bouwwerken zijn hart op te halen.

Buiten de wallen der oude Spaansche veste is een nieuwe stad verrezen met breede, zeer goed onderhouden verkeerswegen, straten en ruime parken. Onder deze laatsten is de Luneta, het geliefde wandelpak der Manilabewoners, wel het voornaamste. Van daar uit overziet men de geheele baai van Manila tot ver in het westen, ontwaart men op den achtergrond het eiland Corregidor, „the sleeping watchdog of the bay" en geniet men in den heerlijke koelte aanwaaienden zeewind des avonds van de schoonste zonsondergangen. Dagelijks worden hier van 6—7 uur door Amerikaansche militaire muziekkorpsen zeer goede concerten gegeven en paradeert daar de voorname Amerikaansche bevolking van Manila in fraaie equipages en auto's, terwijl ook het militaire en burgerelement er rijk vertegenwoordigd zijn.

Een niet minder geliefkoosde plek vormen the botanical gardens. De vrij groote uitgestrektheid gronds, waarop deze gelegen zijn, werd door SEBASTIAN VIDAL aan de stad ten geschenke gegeven, om aldaar een openbaar park te stichten. Hij bracht zelf daar reeds alle mogelijke houtgewassen der Philippijnsche eilanden bijeen en onder het Amerikaansche bestuur is dit, oorspronkelijk in aanleg eenvoudige, park herschapen in een met fraaie wandel-en rijwegen doorsneden verzamelplaats der tropische flora, waaronder waardevolle planten en boomen uit alle oorden der wereld. Ook hier worden openbare muziekuitvoeringen gegeven door den Constabulary Band. Het dankbare nageslacht vereerde SEBASTIAN VIDAL een fraai standbeeld ter plaatse. Sedert korten tijd zijn the botanical gardens ook een zoölogische afdeeling rijk, die evenwel nog slechts weinig beteekent.

Het passarleven is onder de Philippinos even krachtig ontwikkeld als onder onze inlandsche bevolking, daar ook zij de gewoonte hebben slechts de levensbehoeften te koopen, die zij op het oogenblik noodig hebben. De passargebouwen, door de zorgen van het Amerikaansch gouvernement opgericht, zijn zeer goed, ruim en zindelijk. Op de levensbehoeften, die aldaar ter verkoop worden aangeboden, wordt streng toezicht gehouden door beambten van het Bureau of Health. De Divisoria Market, een der voornaamste passars, is een bezoek overwaard, vooral uit een hygiënisch oogpunt.

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Een zeer belangrijke tak van industrie in Manila is het vervaardigen van sigaren en sigaretten, waarvan er jaarlijks vele millioenen, voornamelijk naar Amerika en Australië worden uitgevoerd. De groote afname, die Manilatabak in deze werelddeelen vindt, schijnt de meening, die men hier te lande zoo dikwerf hoort verkondigen, dat dit produkt tegenwoordig niet meer zoo goed is als in vroeger jaren, te logenstraffen. In deze industrie vinden duizende mannen, vrouwen en kinderen een goed bestaan en het bezoek aan de sigarenfabriek Maria Luisa van den Heer MEERKAMP VAN EMBDEN behoort tot de aangename herinneringen van onze reis.

Een 15—20000-tal bewoners van Manila leeft op het water in vaartuigen van allerlei vorm en grootte, bekend onder de namen cascos, lonchas en bancas, die voornamelijk dienen voor het vervoer te water van allerlei handelsartikelen. Niet minder dan de bewoners van het land, is deze waterbevolking verzot op hanengevechten, zoodat onder het vaste personeel van zulk een vaartuig ook meestal minstens één vechthaan behoort. De hartstocht voor deze hanengevechten en het daarmede gepaard gaande dobbelen, vormen te zamen een der voornaamste ondeugden van den Philippino, en worden als een hoofdoorzaak beschouwd van den eeuwigdurenden geldnood, waarin deze steeds verkeert. Vermoedelijk nog meer om deze reden dan wel uit een oogpunt van dierenbescherming, heeft het Amerikaansche gouvernement de hanengevechten ten strengste verboden en zijn zij slechts op enkele bepaalde dagen geoorloofd. Naast de reeds genoemde minder goede eigenschappen heeft de Philippino er nog meer: hij is geen liefhebber van den arbeid en zeer ijdel, verzot op mooie schoenen en in voortdurende bewondering van zijn haartooi, waaraan hij groote zorg besteedt. Hij heeft geen behoefte aan alcohol of opium.

Het klimaat van Manila is tropisch: in het warme, droge seizoen, Maart, April en Mei, is de temperatuur het hoogst:

80.4—83.8 °F., in den regentijd, van Juni tot October, is deze 68—82.4 °F. en in den koelen tijd, November tot en met Februari, 77.2 — 79 °F [1]). De vochtigheidsgraad van de atmospheer is zeer hoog; de avonden en nachten zijn vrij koel.

Met het oog op de typhoons en aardbevingen, die in Manila niet zoo zeldzaam zijn, is het meerendeel der huizen, behalve de onderbouw, van hout. De nieuwere gouvernementsgebouwen worden opgetrokken van gewapend beton.

De sanitaire verhoudingen zijn onder de onvermoeide werkzaamheid van den Director of Health Dr. VICTOR G. HEISER in de jaren van het Amerikaansche bestuur zeer belangrijk verbeterd. Wat op het gebied der hygiëne in Manila is gedaan, verdient onze grootste belangstelling. Ik zal daarom trachten aan het slot van dit verslag daarover een en ander mede te deelen, gedeeltelijk ontleend aan de berichten van het Bureau of Health, gedeeltelijk aan hetgeen ik zelf daarvan heb gezien.

Wij maakten van deze vrije dagen ook gebruik om een bezoek te brengen aan de groote militaire ziekeninrichting van Manila, the Division Hospital, aan het hoofd waarvan een Kolonel [2]) is geplaatst, die ons met niet genoeg te waardeeren welwillendheid gedurende drie en een half uur rondleidde en onvermoeid was om ons alles te toonen, bij welke gelegenheid wij den indruk kregen, dat hij niet alleen in naam de chef was van dit groote hospitaal, maar ook in der daad volmaakt op de hoogte was van alles wat den dienst daarin betrof. Tijdens dat bezoek is werkelijk een gevoel van zeer groote waardeering over mij gekomen voor de wijze waarop de verpleging van den zieken militair tot in de kleinste bizonderheden is geregeld.

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[1]) Deze cijfers geven de gemiddelden aan over de jaren 1880 1906.

[2]) Met opzet vermijd ik overal het noemen van namen, omdat, hoewel mijn dankbaarheid jegens hen, die ik als autoriteiten ontmoette, zeer groot is, ik de vaste overtuiging heb, dat ieder ander in hun plaats dezelfde mate van welwillendheid zou hebben getoond.

Ons bezoek was geheel onverwacht: wij zagen dus den toestand, zooals deze in werkelijkheid is, en geven de verzekering, dat die met het woord „uitstekend" niet overdreven is getaxeerd.

De gebouwen, waarin het hospitaal is gehuisvest, zijn die uit den Spaanschen tijd, en al zou een nieuw gebouwde ziekeninrichting zeker aan andere eischen moeten voldoen dan de tegenwoordige, het is eer te loven dan te laken, dat de Amerikanen, ziende, dat het mogelijk was er nog iets bruikbaars van te maken, dit verkozen, omdat er voor zoo onnoemelijk veel verbeteringen geld noodig was en nog steeds is.

Een ziekenzaal in het militair hospitaal te Manila maakt een heel wat aangenameren indruk dan dit ten onzent het geval is. De kribben zijn alle wit geschilderd en aan het hoofdeinde van iedere krib bevindt zich een keurig kastje, voor het opbergen van allerlei kleinigheden. De paardenharen-matrassen rusten op staaldraadramen, het beddegoed is alles zindelijk en frisch. De zalen zijn voorzien van draadgaasafsluitingen, om alle vliegend ontuig buiten te houden en een aantal electrische waaiers zorgen voor een aangename luchtbeweging. Aan het hoofd van iedere ziekenzaal staat een hoofdverpleegster, hetgeen, daar de Amerikaansche, evenals de Engelsche verpleegsters, gewoonlijk uit de betere klassen van de maatschappij afkomstig zijn, en door de officieren van gezondheid, zoowel als door de ondergeschikten, met onderscheiding worden behandeld, aan het geheel dien beschaafden indruk geeft, die in een ziekeninrichting bezoeker en verpleegde even weldadig aandoet. Onder deze verpleegster doen mannelijke verplegers dienst, niet op bloote voeten in vuile sloffen, maar behoorlijk en net gekleed. Van iederen lijder wordt een ziektegeschiedenis bijgehouden, die in de meest volmaakte orde is: uitgebreide anamnese, curven van temperatuur en pols, resultaat van nauwkeurig urine-onderzoek enz. Telkenmale als een soldaat in het hospitaal is opgenomen, wordt zijn vorige ziektegeschiedenis aan de nieuwe gehecht, zoodat men zich in korten tijd omtrent alles, wat op den lijder betrekking heeft, kan op de hoogte stellen.

De voeding der zieken is uitstekend. In de keukens laat de orde en zindelijkheid niets te wenschen over. Het brood, voor de zieken bestemd, wordt in het hospitaal zelf gebakken; het door een eigen destilleerinrichting gezuiverde drinkwater wordt in buizen door het geheele gebouw geleid en op verschillende plaatsen in het hospitaal zijn drinkwaterreservoirs opgesteld, voorzien van een kraan, waarin het water door ijs wordt koel gehouden. Het destilleeren van het drinkwater was noodig toen Manila dit moest ontleenen aan de oude Spaansche leiding, wier water niet betrouwbaar was. Sedert 1908 is Manila evenwel van een nieuwe waterleiding voorzien, die een dergelijke handelwijze overbodig maakt; men blijft er echter mede voortgaan. Voor huishoudelijk gebruik loopt door het hospitaal een buizenleiding, waarin niet gedestilleerd water aanwezig is; bij iedere kraan van deze leiding staat de waarschuwing te lezen: „Drink no city-water". De baden zijn, behalve die van geneeskundigen aard, zonder uitzondering stortbaden. Andere dan waterclosets zijn onbekend. De operatie-kamers, septische zoowel als aseptische, en de verbandkamers zijn uitstekend ingericht en van het beste op gebied van ziekenverpleging voorzien. De afzonderlijke afdeelingen voor oog-, neus-, keel- en oorziekten bezitten alle een volledig armamentarium, de keurig ingerichte tandheelkundige kliniek, met een doctor dent. surg. aan het hoofd, is uitgerust op een wijze, dat zij de meeste particuliere inrichtingen van dien aard bij ons te lande in de schaduw stelt. Er is een volledig laboratorium voor onderzoek en behandeling met Röntgenstralen. Voor de herstellende manschappen bestaan goede dagverblijven buiten de ziekenzalen, waar ook gegeten wordt. De officierskamers zijn vriendelijk ingericht en tot een home gemaakt; de gelegenheid voor verpleging van echtgenooten van officieren en ook van mindere militairen laat niets te wenschen over. Behalve een goed ingericht laboratorium voor klinische onderzoekingen, is aan het hospitaal annex, maar geheel zelfstandig, een laboratorium voor onderzoekingen op het gebied der tropenziekten.

Ook voor het verplegend personeel wordt uitstekend gezorgd. Wij zagen juist in aanbouw een nieuw verblijf voor de ziekenverpleegsters, waarin zij zich zonder twijfel recht te huis zullen gevoelen. Het verblijf van de hospitaalsoldaten was ook zeer voldoende. Zelfs is er een billardzaal aanwezig, waarin deze hunne vrije uren aangenaam kunnen doorbrengen.

Het ijs, dat in Manila gebruikt wordt, is afkomstig uit de Gouvernements ijsfabriek, waar het natuurlijk van gedestilleerd water wordt bereid. In die fabriek wordt zooveel water dagelijks gedestilleerd, dat er nog genoeg is om als drinkwater aan de bevolking te worden verkocht. Men is in Manila geheel vrij van de ergernis, die ons dagelijks bekruipt, over de meer dan vuile gelegenheden waar het ijs in zoogenaamd zaagsel, dat in werkelijkheid echter niet veel meer is dan straatvuil, in het klein aan de verbruikers wordt geleverd. Terecht heeft de Amerikaan de overtuiging, dat ijs niet uitsluitend een genotmiddel is, maar dat zoowel voor de ziekenverpleging als voor het bereiden van een in de tropen zoo onmisbaren koelen dronk, ijs geworden is tot een levensbehoefte, die de volle aandacht van de autoriteiten verdient.

Een anderen dag gold ons bezoek het Fort Mac Kinley, dat ten onrechte dien naam van fort draagt, daar het in het geheel geen fort doch een kamp is, waar de hoofdmacht der Amerikaansche troepen is gestationneerd en zich ook de ambulancemagazijnen bevinden. Aan een bespreking van hetgeen wij hier gezien hebben, wil ik mij liever niet wagen, uit vrees mij te begeven op een terrein, waar ik niet geheel meer thuis ben.

De militaire gevangenis, in de onmiddellijke nabijheid gelegen, werd ook door ons bezocht en zelfs daar liet de zorg voor de hygiëne niets te wenschen over. De gebouwen zijn ruim, rein, frisch en voorzien van stortbaden en waterclosets. De voeding leek mij beter dan ik die in eenige gevangenis ooit zag.

Op Zaterdag den 5en Maart, des namiddags te drie uur, werd in het Administratie-gebouw van het nog niet geheel voltooide Philippine general hospital de openingszitting van het Congres gehouden. Het gebouw was aan de buitenzijde sierlijk getooid met de vlaggen der verschillende natiën, die afgevaardigden hadden gezonden. Onze driekleur prijkte daar fier en trots aan de rechterzijde van de Stars and Stripes en had alzoo de eereplaats.

De Vice-Gouverneur-Generaal Z. E. NEWTON W. GILBERT, die, wijl zooals ik boven reeds opmerkte, de Gouverneur-Generaal zelve voor dienstzaken op reis was, de openingsrede zou houden, werd door een plotseling opgekomen ongesteldheid daarin verhinderd. In zijn plaats heette een der Philippijnsche Commissioners, een ambt, naar ik vernam, ongeveer gelijkstaande met dat onzer leden van den Raad van Nederlandsch-Indië, de Congresleden welkom in goed Engelsch, doch met een duidelijk merkbaar Spaansch accent. Daarna sprak de burgemeester van Manila FELIX M. ROXAS een vurige Spaansche rede uit, waarvan ik niets heb verstaan en ten slotte nam de voorzitter van het congres Dr. PAUL C. FREER het woord en las op saaien toon een vrij droge verhandeling voor over The tropical sunlight, waarin vooral de goede eigenschappen van het tropische zonnetje niet werden vergeten, zeker om ons te troosten over de smelthitte, waarin wij op dit voor ons ongewone uur van den dag genoodzaakt waren hem aan te hooren. Na afloop dezer plechtigheid werd het nieuwe hospitaal bezichtigd, dat, voor zoover daarover op dat oogenblik reeds een oordeel kon worden uitgesproken, een modelinrichting belooft te worden, waarin met alle eischen der ziekenhuishygiëne is rekening gehouden. In enkele lokalen en gaanderijen was bovendien een vrij onbeteekenende tentoonstelling opgesteld van pathologisch-anatomische praeparaten, op verschillende tropische aandoeningen betrekking hebbende, van uit een pathologisch oogpunt schadelijke insekten en van de voedingswaarde der verschillende door de Philippinos gebruikte levensmiddelen. Burroughs Wellcome en Co. stelden een grooten voorraad geneesmiddelen en allerlei handig verpakte onderzoekingstoestellen ten toon.

Des avonds om 9 uur was er „Reception and dance" in de University Club ¹). Wanneer ik niet bij andere gelegenheden de ervaring had opgedaan, dat de Amerikanen ook vroolijk kunnen feestvieren, dan zou ik daar zeker niet tot die ontdekking zijn gekomen. Het ging er zoo saai toe, dat al zeer spoedig door een groot deel der aanwezigen het snoode plan werd beraamd om heimelijk te deserteeren naar de Duitsche Club, waar een gezellige avond gegeven werd ter eere van een Duitsch Eskader, dat Manila bezocht. Daar was het al vreugde en gezelligheid en noode namen wij afscheid, toen het reeds heel laat, of zoo men wil, vrij vroeg geworden was om de noodige krachten te verzamelen voor den tocht naar Canacao, aangeboden door the Medical Corps United States Navy, ten einde het aldaar aanwezige Marine-hospitaal te bezoeken. In een groote stoomsloep staken wij op Zondagmorgen de mooie baai van Manila over. Het hospitaal ligt op korten afstand van het schilderachtig gelegen Cavite, welks vervallen, met mos begroeide wallen, waar de mooie oude Spaansche kerk nieuwsgierig boven uitkijkt, ons de dagen van Mei 1893 in herinnering brachten, toen de kanonnen van Admiraal DEWEY's vloot in de baai van Cavite de Spaansche oorlogschepen in den grond

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¹) Verdere feestelijkheden worden niet vermeld; het waren er vele.

boorden. Nog langen tijd staken gedeelten van de wrakken der gezonken Spaansche schepen hier en daar boven de zeeoppervlakte uit en eerst twee jaren geleden werden deze alle door de Amerikanen opgeruimd. De niet ontredderde schepen der Spanjaarden zijn later ingericht voor kolenschepen en liggen rustig in de baai te droomen van vroegere glorie en vergane grootheid.

Onze vriendelijke en onvermoeide gastheeren toonden ons een ziekeninrichting, waarop de Amerikaansche Marine met recht trotsch mag zijn: godin Hygieia wordt er op de meest voortreffelijke wijze gediend. Het hospitaal is ruim gebouwd en frisch gelegen in de onmiddellijke nabijheid van de baai. In de ruime zalen, die overal met metaalgaas afgesloten en van electrische fans voorzien zijn, getuigen de keurige, wit gelakte ijzeren kribben der lijders, met hun wit gazen klamboes, van reinheid en orde. Malarialijders worden er in een afzonderlijke klamboekamer verpleegd. Ook hier stortbaden en uitsluitend waterclosets met ruime gelegenheid voor het wasschen der handen. Zelf gedestilleerd water in een leiding door het geheele gebouw gevoerd, groote koelkamers met eigen gemaakt ijs, goed ingerichte operatiekamers en onderzoekingslokalen van de beste en nieuwste vindingen op ieder gebied voorzien, een goed laboratorium voor klinische onderzoekingen: dit alles getuigt van de groote waarde door de Amerikaansche Regeering toegekend aan de begrippen der moderne ziekenverpleging, waarvoor haar geen geldelijke offers te groot zijn.

Na een eenvoudige lunch, waarbij de officieren van gezondheid, daarin voorgegaan door hun Chef, de gasten bedienden en van het noodige voorzagen, aanvaardden wij den terugtocht naar Manila, hoogst dankbaar en zeer voldaan over hetgeen wij gezien hadden.

Maandag den 7en Maart des morgens ten 9 ure had de eerste wetenschappelijke samenkomst plaats in hetzelfde

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lokaal, waar de openingszitting gehouden was. Alvorens echter tot de werkzaamheden van den dag werd overgegaan, verkreeg schrijver dezes het woord, ten einde gevolg te kunnen geven aan de hem door de Nederlandsch Indische Regeering verstrekte opdracht: in haar naam het Congres veel voorspoed op zijn arbeid toe te wenschen. Dit bewijs van belangstelling onzer Regeering, de eenige der vertegenwoordigden, die daaraan had gedacht, in het loffelijk streven der Far Eastern Association of Tropical Medicine werd met warme waardeering door de aanwezigen ontvangen, evenals de begroeting namens onze Vereeniging tot bevordering der geneeskundige wetenschappen in Nederlandsch-Indië.

Het ligt niet in mijne bedoeling een volledig verslag te geven van de onderwerpen, die gedurende de verschillende dagen behandeld werden. Ik zou daartoe om meer dan één reden niet in staat zijn. In de eerste plaats niet, omdat aan de voordrachten buitengewoon weinig zorg werd besteed: de meeste werden voorgelezen in een tempo zóó snel en op zóó eentoonige, nagenoeg onverstaanbare wijze, dat waarlijk niet alleen voor de vreemdelingen van vele het grootste gedeelte van den inhoud verloren ging. In de tweede plaats is het zoo goed als ondoenlijk gedurende 7 dagen achteren van des morgens 9 tot des middags half twee wetenschappelijke voordrachten aan te hooren over alle mogelijke onderdeelen der geneeskunde, met de daarbij behoorende discussies, en dan, na vermoeiende tochten in de middaguren, aan het einde van den dag niet volkomen vergeten te zijn, wat men aan het begin heeft gehoord. Het komt mij bovendien voor, dat de organisatie van het Congres te wenschen overliet: het dagelijksch programma was te overladen, de zoogenaamde discussies hadden daarvan maar heel weinig en ontaardden dikwijls in voordrachten langer dan de oorspronkelijke. Ook het toestaan van niet aangekondigde voordrachten bij een reeds zoo uitgebreide lijst van werkzaamheden behoorde niet te geschieden. Doch ik wil zacht zijn in mijn oordeel: alles moet geleerd worden, ook het houden van Congressen en vermoedelijk zullen vele der nu gemaakte fouten bij een volgende gelegenheid vermeden kunnen worden.

Daar alle op het Congres gehouden voordrachten worden afgedrukt in The Philippine Journal of Science, Section B, Medical Sciences [1]), kan de belangstellende die daar in hun geheel vinden. Slechts op enkele wil ik de aandacht vestigen.

Majoor W. P. CHAMBERLAIN of the United States Army deelt in zijn „Statistical studies of Uncinariasis among white men in the Philippines" gegevens mede, die werkelijk voor een belangrijke verspreiding van den Necator Americanus (hookworm) onder de Philippinos pleiten. Niet minder dan 15°/₀ der bevolking werd daarmede behept bevonden. Ph. STRONG vond bij een onderzoek van 4106 Philippino-gevangenen 52°/₀ geinfecteerden. Vermoedelijk is deze darmparasiet in de Philippinen ingevoerd door de Amerikaansche soldaten, want onder deze komt zij in buitengewoon belangrijke verspreiding voor. Chamberlain vond dat 60°/₀ der militairen, afkomstig uit de zuidelijke staten van Noord Amerika (Maryland, Virginia, West Virginia, North Carolina, South Carolina, Georgia, Florida, Alabama, Mississippi, Louisiana, Texas, Kentucky, Tennessee, Arkansas en Missouri besmet waren, en van de nieuw aangeworvenen 85°/₀. Na de eerste drie jaren van hun dienstverband daalt dit cijfer tot 11°/₀, ten gevolge der gunstige hygiënische verhoudingen, waaronder de soldaten leven. Onder de militairen uit de noordelijke staten is het aantal veel geringer, maar toch nog zoo groot, dat volgens CHAMBERLAIN'S meening 25°/₀ van het geheele leger den hookworm herbergt. Het landbouwersleven en het loopen op bloote voeten schijnen voorbeschikkende oorzaken voor de besmetting te zijn: van de onderzochte personen, die vóór hun in dienst treden het landbouwers bedrijf uitoefenden, waren er 81°/₀ geinfecteerd. Gelukkig waren de meeste dezer personen niet ziek, leden niet aan Uncinariasis, maar toch wordt de aanwezigheid van den hookworm in het darmkanal beschouwd als een oorzaak voor de verzwakking van het organisme, dat daardoor voor allerlei andere besmettingen veel toegankelijker wordt. Zooals uit het jaarlijksch rapport van het Bureau of Healh over 1909 blijkt, is dan ook de strijd tegen dezen parasiet met ernst ter hand genomen.

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[1]) De prijs van dit tijdschrift is $ 3, United States currency per jaar, te zenden aan den Director of Printing, Manila. P. I.

De verhoudingen op de Philippijnen met betrekking tot deze hookwormbesmetting komen, zooals blijkt, in velerlei opzichten overeen met datgene, wat door verschillende onderzoekers in onze koloniën voor het Anchylostomum duodenale is aangetoond. Of de hookworm in deze gewesten voorkomt is mij niet bekend, evenmin of er misschien onder de Philippijnsche hookwormen niet vele anchylostomen waren. Het onderscheid is niet zoo bizonder groot, maar toch wel de moeite waard gemaakt te worden.

Zeer bizondere aandacht verdient op de Philippijnen de buitengewoon groote kindersterfte: 60°/₀ van alle sterfgevallen betreffen kinderen beneden de 5 jaren. Uit het reeds boven door mij genoemde rapport van het Bureau of Health blijkt b.v. dat in Manila, met in 1909 een bevolking van 223 542 inwoners, 8685 geboorten plaats hadden en 4600 sterfgevallen van kinderen beneden het jaar. d.i. 52°/₀ van alle geboren kinderen. Dit verschijnsel maakte natuurlijk ook op het Congres een onderwerp uit van meer dan een voordracht. Als hoofdoorzaken voor die groote sterfte worden genoemd malaria, beri-beri en de hoogst onvoldoende voedende eigenschappen der moedermelk, welk laatste hieruit blijken moet, dat naar verhouding de sterfte onder de borstkinderen veel grooter is dan onder de kunstmatig gevoede. Of die diagnose van beri-beri bij kinderen beneden het jaar juist is, meen ik zeer ernstig te moeten betwijfelen, de daarvoor aangevoerde gronden bewezen m.i. niets meer dan dat de kinderen leden aan ondervoeding, maar niet aan de gevolgen eener polyneuritis. Dat de Amerikaansche Regeering het gevaar dezer groote sterfte voor haar koloniën inziet, het bevolkingscijfer gaat eer achter dan vooruit, blijkt uit het feit, dat voor het jaar 1910 een bedrag van $ 10.000 is uitgetrokken om die vereenigingen, wier doel een vermindering van de kindersterfte beoogt, geldelijk te steunen.

Door de Drs. CLEGG en WHITMORE werd weder eens een zuurvaste bacil vertoond, in symbiose met amoeben gekweekt uit een leproom, die zonder eenigen anderen waarborg dan de genoemde voor den leprabacillus werd aangezien. Proeven, met sera en vaccins met dezen leprabacillus bereid, genomen op lepralijders, bleven zonder eenig gevolg. Maar men geeft den moed niet op. Het is zeer wel mogelijk, dat men hier werkelijk met den leprabacil te doen heeft, doch, naar het mij voorkomt, dient dit nog nader te worden bewezen en alvorens dit bewijs is geleverd, gelijken proefnemingen tot bereiding van specifieke geneesmiddelen niet op ernstig wetenschappelijk werk.

Het doet mij groot genoegen te kunnen boekstaven, dat Collega H. M. NEEB met zijn mededeeling over The Parthenogenesis of the female crescent-body het succes had, dat deze belangrijke ontdekking ten volle verdient. Door Dr. W. T. DE VOGEL was een rapport over Myzomyia Rossii as a Malaria-carrier ingezonden, dat door den Secretaris Dr. FRANCIS CLARK werd voorgelezen op een wijze, dat het mij zeer zou verwonderen, wanneer een der aanwezigen er een enkel woord van heeft verstaan.

De meest belangrijke dag was ontegenzeggelijk die van Vrijdag 11 Maart, op welken niet minder dan 5 voordrachten gehouden werden, die betrekking hadden op de aetiologie

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van beri-beri. Ik kan die onmogelijk hier in extenso alle bespreken, doch verwijs de belangstellenden naar de reeds bovengenoemde publicatie. Gelukkig hadden de goden het zoodanig beschikt, dat ik het eerst aan het woord kwam en daardoor gelegenheid had te toonen hoeveel belangrijk pionierswerk op het gebied van deze ziekte, met betrekking tot haar aetiologie, prophylaxis en genezing, door Nederlandsche onderzoekers, sedert EYKMAN'S belangrijke ontdekking van de polyneuritis bij hoenderen en de beteekenis van het zilvervlies, zoowel binnen als buiten het Geneeskundig Laboratorium is verricht. Met opzet was door mij dit onderwerp gekozen en gegoten in den vorm, waarin ik het voordroeg, omdat maar al te veel, nu van zoovele zijden erkend wordt, dat beri-beri een ziekte der rijsteters is, de verdiensten der Nederlandsche onderzoekers op dit gebied worden voorbijgezien.

Alle sprekers waren het er over eens, dat in het voortdurend gebruik van geheel afgewerkte rijst (clean = white = polished rice) de hoofdoorzaak moet werden gezocht voor het ontstaan der beri-beri. DR. HANS ARON of the Philippine medical school meent uit zijn onderzoekingen te mogen afleiden, dat het hoofdzakelijk zijn de Ph. verbindingen, die met het polijsten van de rijst verdwijnen. De vorm waarin Ph. in het zilvervlies voorkomt, is die van Phytin = anhydro-oxy-methylen-diphosphorzuur, een oplosbare stof, die door de Gesellschaft für Chemische Industrie te Basel in den handel is gebracht. Slechts weinige sprekers oonden bij de discussies aanhangers te zijn van een infectietheorie, waarin de geheel afgewerkte rijst als causa remota dienst deed, zoo o. a. CASTELLANI (Ceylon) en SHIBAYAMA (Japan).

In een daarvoor door het Gouvernement beschikbaar gestelden extra-trein, vertrokken wij Zaterdag 13 Maart des avonds ten 10 ure naar Baguio, gelegen in het Benguetgebergte op een hoogte van 5000 voet, op 170 Mijlen afstands van Manila. De tocht daarheen gaat per spoor tot Camp. No. 1 voorbij Dagupan, waar wij den volgenden morgen ten 7 ure aankwamen en van daar per automobiel over den prachtigen bergweg, aan wiens aanleg reeds ± 5000000 dollars werden ten koste gelegd. Deze weg, zeker een der schoonste bergwegen, die met het moderne vervoermiddel kunnen worden bereden, kronkelt zich door kloven en over bergpassen (ongeveer 200 bruggen), langs bergstroomen en watervallen naar den in het hart van het gebergte van Noord-Luzon gelegen „Summer Capital of the Philippine Islands", met zijn heerlijk frisch klimaat, zijn prachtige natuurtafreelen en zijn rijken schat van pijnboomen en fraaie bloemen. Zoo ergens, dan kan zeker hier herstel gevonden worden van de krachten, die in het uitputtende leven, door de Amerikanen in dit tropische land geleid, noodzakelijk spoedig moeten verloren gaan. Baguio is dan ook het sanatorium, waar ieder ambtenaar jaarlijks voor minstens 14 dagen heen wordt gezonden. Bestaat voor dezen of genen de wenschelijkheid dien tijd te verlengen, dan kan dit geschieden en wordt zulk een ambtenaar tijdelijk belast met werkzaamheden op een der aldaar aanwezige Gouvernements bureaux. Zorg voor een onderkomen behoeft de naar Baguio gezondene niet te hebben, want het Gouvernement bouwde er zeer goede woningen, waarin hij zijn intrek kan nemen. In Juli 1909 werd er een hospitaal geopend voor 44 lijders, een inrichting, die een lust is voor het oog om te aanschouwen, en dit jaar zou er een gelegenheid tot stand komen voor de verpleging van lijders aan tuberculose. Het militaire departement is er vertegenwoordigd door een goede kazerne, uitstekende woningen voor officieren met hun gezinnen en een net ingericht militair hospitaal te Camp John Hay. Ook voor onderwijs wordt er gezorgd. Voor den particulier, die van het heerlijke bergklimaat wil genieten, bestaan er twee goede hotels. Niets wordt

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achterwege gelaten om het leven er mogelijk en aangenaam te maken. Nu reeds zijn er prachtige wegen aangelegd, en parken met de heerlijkste bloemen nooden tot een wandeling, die in de schoonste panorama's haar belooning vindt. Een poloveld ontbreekt natuurlijk niet. Behalve het op een zeer schilderachtig punt gelegen buitenverblijf van den Gouverneur-Generaal, verrezen er in korten tijd een aantal gouvernementsbureaux, voorloopig uit hout opgetrokken, doch met de bedoeling om geleidelijk door steenen gebouwen te worden vervangen. Bovendien bevindt zich aldaar The Mirador Observatory, het centrale meteorologisch observatorium van Manila. Op slechts korten afstand van Baguio, te Trinidad, liggen de gouvernements landbouwproefstations, waar de beste Europeesche groenten worden gekweekt. Een groot bezwaar is op het oogenblik nog de reis van Manila naar Baguio, maar ook daarin zal worden voorzien, want het plan bestaat een electrische baan aan te leggen over de bergen, die in één rechte lijn deze beide punten verbindt. In een causerie, die de Secretary of the Interior Department WORCESTER te Baguio voor de congresleden hield, betoogde hij, dat het Amerikaansche Gouvernement zich al die geldelijke opofferingen gaarne wil getroosten, overtuigd als het is in de verhoogde arbeidsintensiteit, die het gevolg is van het verblijf in zulk een gunstig klimaat, deze weer ruimschoots terug te winnen. Dat is praktische hygiëne, die van geheel andere opvattingen getuigt dan in onze koloniën worden gehuldigd, waar de enkele honderde guldens gouvernementssubsidie, door sommige sanatoria genoten, onder invloed eener verkeerde bezuinigingswoede werden ingetrokken.

Een groot gedeelte van het programma, voor den dag van aankomst te Baguio vastgesteld, viel in letterlijken zin in het water door den hevigen regen, die in den namiddag begon te vallen en van geen ophouden wist. Den volgenden morgen werden er nog slechts een paar voordrachten gehouden in de Governor-General's office, waar het grootste gedeelte van den tijd werd besteed aan de bespreking der conclusies, die, door daartoe op den 1en dag van het congres benoemde commissies samengesteld, aan het oordeel van het congres werden onderworpen. Voor behandeling der volgende vraagstukken waren zoodanige commissies benoemd: beri-beri, lepra, tuberculose, quarantaine, opium en pest. Collega NEEB maakte deel uit van de commissie voor lepra, opium en pest, ondergeteekende van die voor beri-beri, quarantaine en lepra, van welke laatste hij als voorzitter fungeerde. Ik geef hier den tekst der conclusies weer, zooals die na rijp beraad werden vastgesteld, en waarvan gedrukte exemplaren zijn verzonden aan de verschillende mogendheden, die zich op het Congres hebben doen vertegenwoordigen.

„The Congress was attended by seventy-six medical men „engaged in professional work in the Far East and included „official delegates from the Governments of India, Ceylon, „Siam, Netherlands-India, Federated Malay States, Straits „Settlements, Hongkong, Philippine Islands, Tsingtsau, (Im-„perial German Government) and Japan.

„Resolved:

„**Beri-Beri.** That in the opinion of this Association „sufficient evidence has now been produced in support of „the view that Beri-Beri is associated with the continuous „consumption of white (polished) rice, as the staple article „of diet, and the Association accordingly desires to bring „this matter to the notice of the various Governments „concerned".

„**Tuberculosis.** That the Far Eastern Association of Tro-„pical Medicine should use its influence to cause the for-„mation of a national anti-tuberculous society in each „political entity represented in the Far Eastern Association „of Tropical Medecine. These societies to be formed „along the lines of existing anti-tuberculous societies in

„other parts of the world, yet revised to facilitate the „work under local conditions".

„Lepra.

„1. Lepra is to be regarded as a dangerous communicable disease.

„2. Compulsory notifications of all cases of leprosy to „the authorities is essential.

„3. Compulsory segregation of all cases of leprosy is „necessary and preferably in special colonies constructed „for the purpose.

„4. The entrance of aliens afflicted with leprosy into a „country must be prohibited.

„**Quarantine**. That the Far Eastern Association of Tropical Medicine appreciating the benefit which would accrue „from concerted sanitary action on the part of Eastern „Governments, be empowered officially to approach the „following Governments, namely: Philippine Islands, Japan, „Hongkong, French Indo-China, Siam, Netherlands-India, „Straits Settlements and Ceylon, with the view, if possible „of obtaining their official support on the following lines:

„1. To have a common standard for the term „epidemic", „when making reports to, or imposing quarantine against, „each other.

„We suggest the following definition for consideration;

„Plague, Cholera, Small-pox, or Yellow Fever shall be „considered to be epidemic, when, after the first telegraphic „report of its occurrence, any weekly report thereafter shall „show the occurrence of an avarage daily number of three „cases".

„2. To agree to notify each other's territories as infected, „only when the infectious disease shall have assumed epidemic proportions as defined above; and automatically to „withdraw such notification when the avarage number of „cases for three successive weeks has fallen below the „status epidemicus as bove defined.

„3. To circulate weekly returns of Plague, Cholera, „Small-pox or Yellow Fever amongst each other, and also „a telegraphic report on the first occurrence of any of these „diseases, in a clean port or territory.

„4. To insist on a Bill of Health being carried by all „ships, leaving a country declared to be infected, which „intend to proceed to the port of another signatory; such „Bills of Health to include a return of infectious disease „for the previous 48 hours.

„5. To report by telegram to the country concerned, the „departure of an infected or suspected ship (as defined „by the Paris Convention) which may intend to proceed „to any port in the territories of another signatory; and to „endorse the Bill of Health of the said infected or suspected ship with a full account of measures taken to „disinfect or otherwise deal with the said vessel.

Report:

„**Plague.** In our opinion the only practical measure „concerning Plague that we can recommend is the advisability of each country represented agreeing to notify to „other countries the occurrence of cases of Plague within „their borders, the first case by telegram and afterward „by weekly returns.

„**Opium.** Your Committee, appointed to consider the „opium question, regret that the time at their disposal precludes the possibility of giving the subject adequate „consideration.

„They have reviewed the work done and the resolutions „agreed on by the International Opium Commission, Shanghai, 1909.

„With these resolutions your Committee are in agreement, „but it is to be regretted that the paucity of medical commissioners prevented the question being delt with fully „in its medical aspects.

„Any measures which are introduced for the control of

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„opium must be adequate to guard against the danger of „secret remedies containing opium and opium derivatives „being substituted.

„They would suggest that the delegates from the various „countries represented at this conference should be asked „to arrange that the regulations controling the sale and use „of opium in their respective countries, as well as any „observations they may care to make on this subject, be „submitted to the next meeting of this Association in 1912".

Ook werd de wenschelijkheid besproken, ten einde beter dan dit tot nog toe kon geschieden, kennis te kunnen nemen van hetgeen in de verschillende landen in het verre Oosten op tropisch geneeskundig gebied wordt gewerkt, de desbetreffende publicaties in één tijdschrift samen te brengen. The Philippine Journal of Science, dat onder redactie van DR. PAUL C. FREER door het Bureau of Science of the Philippine Government wordt uitgegeven, verklaarde zich tot plaatsing van zoodanige publicaties bereid, daarbij aan iederen schrijver het recht latende zijne mededeelingen eerst te doen opnemen in eigen nationale tijdschriften. Het gebruik van de Engelsche taal is gewenscht.

Tot Voorzitter van de Association voor de eerstvolgende twee jaren, en daarmede ook tot President van het tweede Congres, werd gekozen DR. J. H. ATKINSON, en tot algemeen secretaris-thesaurier herkozen DR. FRANCIS CLARK, beiden te Hongkong en dankbaar werd aanvaard de uitnoodiging van het Gouvernement van Hongkong om in 1912 het Congres aldaar te mogen ontvangen. Ik wil er hier meteen op wijzen, dat voor 1914 de hoop gevestigd is op Batavia.

Na afdoening van verschillende huishoudelijke aangelegenheden en de gewone, doch zeer verdiende plichtplegingen jegens het Gouvernement der Philippijnen, Voorzitter en Secretaris van het Congres en allen, die hadden mede-gewerkt tot het welslagen, werd deze eerste samenkomt der FAR EASTERN ASSOCIATION OF TROPICAL MEDICINE, die als een belangrijk feit in de geschiedenis der geneeskunde in het verre Oosten dient te worden aangemerkt, gesloten. In den namiddag werd de terugtocht naar Manila aanvaard, waar wij den 15en Maart des morgens te 3 uren aankwamen. De Gouverneur-Generaal, die inmiddels van zijn dienstreis was teruggekeerd, bood dienzelfden dag den gedelegeerden en het Congresbestuur een lunch aan in zijn heerlijk aan den Pasig-river gelegen paleis en had zijn stoomsloep ter onzer beschikking gesteld, om ons en nog eenige andere deelnemers, die dienzelfden middag vertrekken moesten, naar het stoomschip te brengen, dat in de haven van Manila lag te wachten en waarheen wij door het Congresbestuur uitgeleide werden gedaan. IJdel was de hoop, dat wij daar de verkwikkende rust zouden vinden, die ons gedurende de vermoeiende en inspannende dagen van het Congres zoo in alle opzichten had ontbroken: God Neptunus had besloten, dat het anders zijn zou! Een staart van een typhoon zweepte het water van de Chineesche zee op tot woeste golven, die met ons schip speelden als met een draaitol. En ofschoon wij Hollanders, als echte zonen van een zeevarend volk, moedig stand hielden en niet mede offerden op het altaar van den vertoornden zeegod, waren toch ook wij recht dankbaar, toen wij in den vroegen morgen van den derden dag Hongkongs wonderschoone haven weder binnenstoomden.

Zooals ik in den loop van het bovengeschrevene meedeelde, is door de Amerikanen reeds veel belangrijk werk gedaan in den betrekkelijk korten tijd van hun bestuur over de Philippijnen. Vóór dien telde de bevolking dier eilandengroep ongeveer $6^1/_2$ millioen zielen en was de sterfte zoo groot, dat gedurende jaren het bevolkingscijfer eer achter dan vooruitging: zij wordt geschat op meer dan 50 $^0/_{00}$

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jaarlijks. Alleen aan pokken stierven in de eerste periode van het Amerikaansche bestuur nog jaarlijks 40000 personen. Voor zoover door een nauwkeurig onderzoek kon worden nagegaan, bestond er geen enkele artesische wel. In het water van alle deelen der eilanden werden dysenterie-amoeben gevonden. In Manila, een stad van meer dan 200.000 inwoners, bestond geen rioleering en alle afval van de menschelijke en dierlijke huishouding werd eenvoudig overal daarheen weggeworpen, waar het toevallig terecht wilde komen. Het drinkwater dezer stad was afkomstig uit een rivier, wier oevers door meer dan 10000 menschen bewoond werden en die de rivier zelve voor bad-, waschplaats, privaat en afvoerkanaal voor alle vuil gebruikten, zooals wij dat van onze kali's gewoon zijn te zien. In de stad zelf bestonden meer dan 31 mijlen aan kanalen, die eigenlijk niet anders waren dan smerige open goten, waarheen alle vuil zijn weg vond. Er bestond niet de geringste gelegenheid voor de verzorging van krankzinnigen, die men òf eenvoudig liet rondloopen, òf wanneer zij gevaarlijk waren, met een ketting aan een paal vastlegde. In het hospitaal San Lazaro, een oud klooster, en in de Palestine Leper Colony werden ongeveer 200 lepralijders verpleegd; de anderen liepen vrij rond. Lijken werden ten getale van 4 en meer in dezelfde kuil begraven, en, om plaats te maken voor nieuwe, werden de overbljfselen der vorige eenvoudig op een hoop geworpen. Beri-beri, malaria, dysenterie telden hun slachtoffers bij duizendtallen. De cholera ontvolkte geheele streken en maakte alleen in Manila soms meer dan 1000 dooden per dag. Quarantainevoorschriften ontbraken geheel, zoodat telkens weer op nieuw de cholera van buitenaf werd ingevoerd en ook de pest herhaaldelijk een aantal slachtoffers maakte.

Reeds zeer spoedig, nadat de Amerikanen dezen desolaten boedel van de Spanjaarden hadden aanvaard, werd met kracht de hand geslagen aan het tot stand brengen van verbeteringen op ieder gebied. Te Manila werd opgericht het reusachtige laboratorium, dat den naam draagt van Bureau of Science en waarvan Dr. Paul C. Freer de directeur is. Aan een grooten staf van medewerkers werd daar het onderzoek toevertrouwd van alle wetenschappelijke vraagstukken op medisch, veterinair, botanisch, zoologisch, anthropologisch en mineralogisch gebied. De groote aantrekkelijkheid van de kennismaking met allerlei nieuwe onderwerpen, leidde tot een breeden stroom van publicaties op ieder gebied, aanvankelijk in verspreide bulletins, later in een afzonderlijk, door het Gouvernement der Philippijnen uitgegeven tijdschrift: The Philippine Journal of Science. De bereiding der dierlijke koepokstof, der verschillende entstoffen en der geneeskrachtige sera werd ter hand genomen, om zich daardoor onafhankelijk te maken van het moederland.

Naast dit Bureau of Science werd opgericht the Bureau of Health, dat voor de stad Manila als stedelijke gezondheidsdienst fungeert, doch tevens de leiding van dezen dienst over de geheele Philippine Islands in handen heeft. Bovendien werden buiten Manila nog ongeveer 300 gezondheidscommissies in het leven geroepen, zoodat de nooden en behoeften op hygiënisch gebied nauwkeurig bestudeerd en in wetgevingen de voorzieningen daartegen konden belichaamd worden. Rioleering, drinkwatervoorziening, verzameling van afval, straatreiniging, afzondering van besmettelijke zieken, dit alles werd ter hand genomen en reeds zeer spoedig was een duidelijke invloed ten goede daarvan merkbaar. In de provincies rondom Manila, die ongeveer één millioen inwoners bevatten, stierven vroeger jaarlijks ongeveer 6000 personen aan pokken: in het jaar 1909 geen enkele. Artesische putten werden bij dozijnen overal geboord en in vele streken daalde na het in gebruik nemen van het artesische drinkwater de sterfte snel van 50—20°/₀₀. De gezondheidsleer wordt in 3500 openbare scholen onderwezen en zelfs het in den aanvang den Amerikanen zoo vijandig gezinde priesterdom helpt mede om onder de bevolking kennis van de eenvoudigste regelen der hygiëne te verspreiden. Door de zorgen van the Bureau of Health, waarvan de energieke Directeur is DR. VICTOR G. HEISER worden bulletins uitgegeven over cholera, dysenterie, tuberculose, kortom over alles, wat op de volksgezondheid betrekking heeft, zoodat een ieder in staat is te bestudeeren welke maatregelen hij heeft te nemen en van overheidswege genomen worden om den algemeenen en persoonlijken gezondheidstoestand te helpen bevorderen. Alleen van datgene, wat in Manila zelf is geschied, hebben wij gedeeltelijk kennis kunnen nemen en ons daarbij kunnen overtuigen van den ernst, waarmede de groote verbeteringen zijn tot stand gebracht, ondanks de buitengewoon groote geldelijke offers, die daarvan het noodzakelijk gevolg zijn.

Op den 1en Juli 1908 werd in Manila de nieuwe waterleiding geopend, die dagelijks 50 milioen gallons [1]) zuiver drinkwater in de stad brengt en aan wier totstandkomen 4000000 dollars zijn ten koste gelegd. Daarnaast beschikt de stad nog over de oude leiding, die 15 millioen gallons water in het etmaal aanvoert, geschikt voor werk- en badwater. Het gebied van de prise d'eau der nieuwe leiding wordt voortdurend door Amerikaansche troepen bewaakt, om elke mogelijk verontreiniging te voorkomen.

Naast den aanleg van een goede waterleiding werd ook onmiddellijk voor den afvoer der faecaliën gezorgd. In den aanvang, en bij wijze van tijdelijken maatregel, werd een stelsel van septic tanks aangelegd en het vraagstuk der rioleering in studie genomen. De lage ligging van Manila maakte de oplossing daarvan niet gemakkelijk. Doch voor geld was dit bezwaar niet onoverkomelijk en ten koste van 3.5 millioen dollars is een rioolstelsel tot stand gebracht, dat gerust eenig in zijn soort kan worden genoemd. Op een diepte, afwisselende van 1.5 tot 5.4 M. is een buizen-stelsel in den grond gelegd, welks doorsneden gaan van 1.75 M. tot 20 c.M. en onder zoodanige helling, dat aan den inhoud een stroomsnelheid is verzekerd van niet kleiner dan 65 c.M. per secunde, wanneer de riolen half vol zijn. Om deze stroomsnelheid over de geheele uitgestrektheid van het rioolstelsel te behouden, zijn in den loop der leiding 5 electrische pompstations aangebracht, die den inhoud der leiding met gering verval van afstand tot afstand overpompen in een nieuw gedeelte met weer grooter verval. Ten slotte verzamelt zich de geheele inhoud in het hoofdpompstation, dat aan het strand gelegen is, wordt daar opgepompt tot een hoogte van 8.8 M. en overgebracht in een 3.05 M. beneden den bodem van de baai gelegen gietijzeren pijpleiding, die op een afstand van 1.5 K. M. van de kust uitmondt. Deze pijpleiding eindigt in een verticaal stuk, met het doel om den uitlaat te doen plaats hebben boven den bodem van de baai. Dit verticale stuk is opgesloten in een kolossaal blok van gecementeerd metselwerk, dat op een houten platform, door een aantal pijlers gedragen, rust. Over de geheele leiding verspreid is een aantal, op de waterleiding aangesloten, tanks aangebracht, ieder van 132 Liter inhoud, waardoor het mogelijk is telkenmale de leiding schoon te spoelen. De electrische pompen, die den rioolinhoud van een lager gelegen gedeelte der leiding in een hooger gelegen gedeelte overpompen, werken geheel automatisch, doch kunnen ook opzettelijk in werking worden gesteld.

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[1]) 1 gallon = 4.54 Liter.

Voor die gedeelten van de stad, waar het voorloopig niet uitvoerbaar is aansluiting aan het rioolstelsel tot stand te brengen, b. v. kleine huisjes en kampongs, zijn onder streng toezicht geplaatste gemeenschappelijke privaten gebouwd, met tonnenstelsel. De tonnen worden geregeld des nachts verwisseld door de zorg van den gezondheidsdienst en de volle vervoerd naar het eindpompstation, waar hun inhoud ook naar zee wordt geleid.

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Zooals ik reeds boven mededeelde werd de breede en diepe gracht, die Manila intra muros omspoelde, gedempt en werden ter plaatse parken en wegen aangelegd. De 31 K. M. sloten en kanalen werden uitgebaggerd en gereinigd van al het vuil, dat zich gedurende eeuwen daarin had opgehoopt, en worden door geregeld baggeren op profiel gehouden.

De straatreiniging en de besproeiing der openbare wegen, niet door middel van koelies, die wel zorg dragen, dat, waar één druppel water is neergevallen, niet nog een tweede komt, maar met groote, door krachtige ossen getrokken sproeiwagens, laten op het oogenblik niets te wenschen over.

Een openbaar slachthuis is gebouwd, the Matadero, van waar het vleesch in afzonderlijk daarvoor bestemde wagens van gemeentewege naar de markten wordt vervoerd. Op den goeden toestand waarin deze verkeeren en het daarop uitgeoefende toezicht, vestigde ik reeds boven de aandacht.

Ter vervanging van de slechte particuliere ziekeninrichtingen uit den Spaanschen tijd werd een hospitaal gebouwd met 160 bedden voor burgerlijke beambten, terwijl dit jaar het ook reeds genoemde nieuwe General Hospital in gebruik zal worden genomen. Een krankzinnigengesticht voor 500 verpleegden plaats aanbiedende is gereed. Evenzeer een keurig ingericht hospitaal voor besmettelijke zieken en in de nabijheid van de bovengenoemde San Juan Bridge, buiten Manila gelegen, is reeds het terrein beschikbaar waar een gelegenheid zal worden geschapen om de lijders aan tuberculose te verplegen en deze zoodanig hygiënisch op te voeden, dat zij voor hun omgeving in de maatschappij geen gevaar meer opleveren.

Een uitgebreide gezondheids-inspectie-dienst werd ingevoerd, staande onder den Assistant-director of Health. Om een goede uitoefening van dien dienst mogelijk te maken is Manila verdeeld in 5 districten, die gezamenlijk weder in 62 onderdistricten zijn verdeeld. In ieder district is, onder de bevelen van een medical-inspector, een station, waar onmiddellijk geneeskundige hulp kan verkregen worden en is een ziekenwagen gestationneerd, om zoo noodig zieken over te brengen naar een hospitaal. Aan twee dezer stations is dit ziekenvervoermiddel een auto, aan de drie andere zijn nog paardenwagens, die evenwel ook geleidelijk door auto's vervangen zullen worden. Bovendien is daar steeds aanwezig een desinfectie-brigade, voorzien van paard en wagen, waarin alle benoodigdheden voor de desinfectie worden vervoerd.

In het geheel bestaat de inspection-division, behalve uit den reeds genoemden Assistant-Director of Health, uit 19 medical-inspectors, 8 municipal physicians, 15 sanitary-inspectors, 85 assistant-sanitary-inspectors and sanitary police, 1 chief-desinfector, 1 assistant-chief-desinfector en 7 assistant-desinfectors. Om ten allen tijde over een voldoend aantal geoefende inspectors en desinfectors te beschikken, wordt de politie ook onderwezen en geoefend, zoodat in geval van nood tijdelijk uit dat corps in de behoefte kan worden voorzien. De inspectors beschikken, om tijd uit te winnen, over het gebruik van een motorfiets.

In de Ordinance No. 86 van 20 October 1906: „An ordinance relating to sanitation and providing a sanitary code for the city of Manila", bij welke alle vorige besluiten buiten werking zijn gesteld, is de wijze, waarop de saniteitsdienst is geregeld en de onderwerpen, waarover deze zich uitstrekt, nauwkeurig omschreven. Tot in welke onderdeelen deze ordonnantie afdaalt, moge b. v. blijken uit het volgende. In Hoofdstuk X, handelende over de barbiers, is bepaald in artikel 114: „Personal neatness.

„Every barber when engaged in any operation of his „trade shall wear a clean white shirt or coat, and shall „keep his fingernails short and well trimmed and he „shall thoroughly wash and cleanse his hands before „operating on any person". Het haarknippen onder den asamboom behoort in Manila tot het verleden.

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Door de Sanitary Inspectors wordt eenmaal in de 3 dagen in tijden van cholera iederen dag, ieder huis geïnspecteerd. In elk huis is op een zichtbare plaats een kaart opgehangen van nevensgaand model, waarop de inspecteerende ambtenaar zijn aanmerkingen plaatst, tevens lastgevende, dat hetgeen niet in orde wordt bevonden, in orde moet worden gebracht; op het niet nakomen van welke lastgeving strafbepalingen zijn gemaakt. Aan de keerzijde van die kaart staat de Spaansche tekst.

Het is natuurlijk niet mogelijk in verdere bijzonderheden af te dalen over alles wat op hygiënisch gebied op Manila is tot stand gebracht, vooral ook met betrekking tot de groote verbeteringen in de kampongs. Voor een tweetal onderwerpen meen ik echter nog de aandacht te mogen vragen, n.l. de lepraverpleging en het gevangeniswezen.

De lepra op de Philippijnen dagteekent, naar het schijnt, uit het begin van de 17e eeuw. Op den 8en Juni 1632 ontving de koning van Spanje een mededeeling van den Captain-General of the Philippine Islands, waarin deze berichtte, dat 144 bekeerde christenen, door den Keizer van Japan gezonden, in de baai van Manila waren aangekomen. De koning gaf last, dat deze bekeerde christenen in de stad zouden worden toegelaten en dat hun een feestelijke ontvangst zou worden bereid, tot welk doel Z. M. behalve de reeds door den Captain-General daarvoor uitgetrokken 500 realen, nog 200 realen extra beschikbaar stelde. Deze bekeerde christenen bleken bij nadere kennismaking lepralijders te zijn, waarvan men zich in Japan wenschte te ontdoen. Sedert heeft zich de ziekte over de eilanden uitgebreid.

Voordat de Amerikanen op de Philippijnen heer en meester waren, werd door de [illegible] lepra die dit wenschte [illegible] toezicht van [illegible]. De [illegible] zoals boven ook reeds is [illegible] en de ziek

B. OF H. No. 37.

BUREAU OF HEALTH FOR THE PHILIPPINE ISLANDS

SANITARY REPORT

TO BE POSTED IN A CONSPICUOUS PLACE

Hour	Inspector.	Condition of—					Has there been any new Unauthorized Construction Since Last Inspection?	Have there been any Cases of Sickness Since Last Inspection?	Remarks
		Closet.	Garbage and Refuse Cans.	Kitchen.	Drains.	Stable.			

The owner of this house is Mr. .... No. ....
t .... District of .... who is respon-
for the structural condition and repair of the premises.

The { care taker / tenant } is ...., who is responsible
the cleanliness of these premises.

NOTE.—In order to be relieved of the responsibility the tenant or care taker
ld notify the health station at No. .... Street .... upon
g up these premises.

Any person who shall deface, alter, falsify, remove, or destroy this sanitary notice,
upon conviction be guilty of a violation of section 666, Revised Ordinances, City
anila.

Please notify the Bureau of Health of any discourtesy on the part of the Sanitary
ectors.

breidde zich meer en meer uit. Doch het vraagstuk der verplichte aangifte en gedwongen afzondering maakte al spoedig een onderwerp van studie uit voor den Amerikaanschen gezondheidsdienst en beide maatregelen werden ingevoerd voor iedereen, zonder aanzien van rang of stand. In de Medical Record van 8 Juni 1907 schrijft Dr. Victor G. Heiser daarover het volgende, woorden, die ik gaarne onderschrijf: „This policy may seem cruel and heartless, „especially to those who are directly affected bij its operation. Isolation is for the best interests of all concerned, „for the benefit of the patients and for the protection of „the public. But, unfortunately, this is a case where the „ends of science and sentiment diverge".

Op den 28^{en} Mei 1906 werd in gebruik genomen de lepra-colonie op het eiland Culion, op 36 uren stoomens afstand van Manila gelegen, waarheen, langzamerhand alle lepralijders zullen worden overgebracht. Op dien datum kwamen 365 lijders, die den vorigen dag het San Lazaro Hospital te Cebu hadden verlaten, op het eiland aan, waar zij door 4 liefdezusters van de orde van St. Paul de Chartres en een priester van de orde der Jezuiten werden ontvangen. Zij waren aangenaam verrast en zeer tevreden over de voorzieningen, die voor hen waren getroffen.

Sedert het werk van de afzondering der lepralijders is ter hand genomen, in 1906, tot einde 1909, zijn 3990 lijders opgespoord. Aan het einde van 1909 waren er op Culion 1926, maar nog niet alle lijders op de Philippijnen aanwezig, zijn overgebracht. Het tegenwoordige aantal wordt geschat op 2273, zoodat er nog ± 350 voor afzondering overblijven. Van deze zijn er echter ± 200 opgenomen in het San Lazaro Hospital in Manila, voor wetenschappelijke studie. Want men vergenoegt zich niet alleen de arme lijders af te zonderen, doch tracht ook de middelen te vinden, die genezing of stilstand der ziekte kunnen bewerkstelligen. Van deze middelen bleek het Nastin, gedurende $1^1/_2$ jaar eproefd, zonder eenig nuttig effekt. Omtrent den invloed er X — stralen en van de Chaulmoogra-olie zegt Dr. Heiser: The great improvement which has taken place in many cases from the use of the X — ray and more especially from the use of crude chaulmoogra-oil, as recommanded by Professor Dyer of New Orleans, has renewed hope to a marked extent". Uit de medegedeelde cijfers blijkt, dat edert de verplichte afzondering het aantal lepralijders met en totaal van 1717 is verminderd.

Het leven van den lepralijder op Culion is dat van den colonist. Zij hebben hun eigen, door henzelf gekozen estuur, houden zich bezig met landbouw en veeteelt en ie werkzaamheden, welke in het belang der kolonie noodig zijn. Van tijd tot tijd kunnen zij door hunne familieeden worden bezocht, en naar mij verzekerd werd heerscht onder de kolonisten in het algemeen een zeer tevreden stemming.

Manila bezit één groote gevangenis, die een oppervlakte van ± 17 acre (1 acre = 4840 vierkante yards = 4047 M^2), waarop ongeveer 50 gebouwen staan. Deze gevangenis is de grootste onder contrôle van het Amerikaansche Gouvernement en misschien de grootste van de wereld. Er is plaats voor 5000 gevangenen, hoewel het gemiddeld aantal aldaar verblijf houdende veroordeelden niet meer bedraagt dan 3000, daar de gevangenen ook in andere deelen der Philippijnsche eilanden kunnen worden te werk gesteld. De gevangenis is geen cellulaire, maar zij is verdeeld in een aantal paviljoens, die ieder plaats hebben voor ongeveer 300 personen, over verschillende slaapzalen verdeeld, alwaar zij onder toezicht staan van uit de gevangenen zelf gerecruteerde oppassers, die door hun goed gedrag een zoodanige onderscheiding verdienen. Na het bezoek aan den Bilibid Prison kwamen mij de woorden in de gedachte, die ik in mijn studententijd zoo menigmaal had gelezen boven

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de fraaie antieke gevangenispoort aan den Heiligen Weg te Amsterdam (sedert herschapen in een badhuis): „Ik wreek geen kwaad, doch dwing tot goed; streng is mijn hand, maar zacht is mijn gemoed". Want dit moet wel de leidende gedachte geweest zijn van the Bureau of Prisons bij de samenstelling der reglementen, op de verpleging der gevangenen betrekking hebbende. De bedoeling van deze is toch niet alleen den misdadiger te straffen voor het door hem begane vergrijp, maar om hem tijdens zijn verblijf in de gevangenis op te voeden tot een goed burger en hem voor te bereiden om na zijn ontslag een goede plaats in de Maatschappij te kunnen innemen. Daarom wordt niet alleen groote zorg besteed aan zijn voeding, die uitstekend is, maar wordt hij ook genoodzaakt reinheid te betrachten voor eigen lichaam en voor zijn verblijf. Bovendien wordt hij gewend aan geregelden arbeid, afgewisseld door rust en ontspanning, zoowel voor het lichaam als voor den geest. Tot het laatste doel werken mede onderwijs, goede lectuur, dagelijksche muziekuitvoeringen door een uitstekend muziekkorps, bestaande uit gevangenen onder een ambtenaar-kapelmeester, voordrachten opgeluisterd met lichtbeelden enz.

De gezondheidstoestand der gevangenen was niet altijd zoo gunstig als die tegenwoordig is. Toen de Bilibid Prison nog onder beheer stond van de justitie, nam de sterfte van jaar tot jaar toe en bereikte zij zelfs een cijfer van 238 $^0/_{00}$. Daarna werd deze geplaatst onder the Bureau of Health. De gewone sanitaire maatregelen, als vermindering van de bevolking, verbetering van drainage en faecaliën-afvoer, regeling van het dieet naar wetenschappelijke gegevens, slaagden er in 6 maanden in de sterfte te doen verminderen tot ± $70^0/_{00}$, maar verder kwam de vermindering niet. Het scheen, dat er factoren in het spel waren, die men tot nog toe buiten beschouwing had gelaten. Van deze onderstelling uitgaande, werd een aanvang gemaakt met het systematisch onderzoek van de ontlasting van alle 3500 gevangenen.

Het resultaat van dit onderzoek was, dat 84°/₀ van hen dragers werden bevonden van minstens 1 darmparasiet, 50°/₀ hadden twee of meer soorten, 20°/₀ drie of meer, en 52°/₀ hadden haakwormen. Na afloop van het onderzoek van één brigade (300 man), werd deze onmiddellijk onder behandeling genomen om de ingewandsparasieten te verdrijven, en reeds spoedig bleek, dat daarna het sterftecijfer in deze brigade kleiner was dan in de niet behandelde afdeelingen. Nu werd de behandeling tot alle gevangenen uitgebreid, met het gevolg, dat het sterftecijfer is gedaald tot 13°/₀₀ en sedert zoo is gebleven. Het schijnt, dat de aanwezigheid van veel parasieten in den darm het weerstandsvermogen van hun drager zoodanig vermindert, dat deze een gemakkelijke prooi wordt voor allerlei besmettingen.

Wanneer de gevangene in de gevangenis wordt opgenomen, wordt hem te kennen gegeven, dat het de plicht is der beambten om voor zijne belangen zorg te dragen en dat het zijn welbegrepen eigenbelang is zich te onderwerpen aan de reglementen en voorschriften en zijn tijd zoo nuttig mogelijk te besteden. Hij wordt onderzocht, gebaad, gebertillonneerd, gephotographeerd, geregistreerd en in de gevangenis geplaatst onder toezicht van den geneeskundige, in quarantaine gehouden en in zoo goed mogelijke omstandigheden gebracht. Daarna neemt het eigenlijke strafdepartement van de gevangenis hem over en geeft hem een soort van militaire opvoeding, waarbij hij tevens de gelegenheid heeft zich aan zijne omgeving te gewennen. Ten slotte wordt hij aan het werk gezet.

De dagverdeeling is als volgt: reveille 5.30 a. m.; ontbijt 5.50 a. m.; aan het werk 6.30 a. m.; terugkeer van het werk 11.30 a. m.; middagmaal 11.50 a. m.; siesta van 12.15 p. m. tot 1.45 p. m.; appel 1.50 p. m.; aan het werk 2 p. m.; terugkeer van het werk 4.30 p. m.; avondparade 4.45 p. m.; avondeten 5 p. m.; dagelijksch bad 5.10 p. m.; avondschool 5.30 tot 6.30 p. m.; uitspanning van 6.30 p.m. tot taptoe

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om 8.45 p. m., nachtrust 9 p. m. Hoogst belangwekkend is het om des middags om 4.45 de avondparade bij te wonen, welk schouwspel men geniet van af den hoofdwachttoren in het centrum van het gebouwencomplex gelegen. Op de tonen van de muziekkapel worden daar een reeks vrije- en orde-oefeningen uitgevoerd, die gezien mogen worden. Na afloop daarvan heeft de uitreiking van het avondeten plaats, die door de groote orde, waarmede dit geschiedt, in den tijd van 7 minuten is afgeloopen.

De gevangenen worden verdeeld naar hun gedrag in drie klassen: de eerste is die van hen, welke niet minder dan gemiddeld 90$^0/_0$ goed gedrag hebben, de tweede klasse niet minder dan 80$^0/_0$, en de derde klasse bevat alle, die daar onder blijven. Verder zijn zij ook nog naarmate van hun geschiktheid als werkman in drie klassen gerangschikt.

De geheele bevolking is verdeeld in een aantal groepen, ieder van 24 man. Zulk een groep staat onder een gevangene met 100$^0/_0$ goed gedrag, een aantal van deze groepen staat onder een bewaarder, die weer een hooger geplaatsten beambte boven zich heeft. Het gedrag der gevangenen wordt nauwkeurig nagegaan en in daarvoor bestemde registers opgeteekend. Iederen avond worden deze registers op de bureaux ingeleverd en bestudeerd. Bij herhaalde overtredingen wordt de gevangene geneeskundig onderzocht om na te gaan of daarvoor in eenigerlei geestelijke of lichamelijke afwijking een oorzaak is te vinden, zoo niet, dan wordt hij disciplinair gestraft. Dit stelsel werkt zoodanig, dat 90$^0/_0$ der gevangenen behooren tot de 1e klasse en van dezen nog een aanzienlijk aantal 100$^0/_0$ goed gedrag hebben.

Alle handwerken nagenoeg worden in de ruime werkzalen uitgeoefend en de produkten van dezen arbeid vinden steeds koopers.

Gevangenen, die door hun goed gedrag daarvoor in aanmerking komen, kunnen verzoeken om plaatsing in de

Iwahig Penal Colony, gelegen op het eiland Palawan in de baay van Puerto Princesa, alwaar zij in landbouw-industrie onderwezen en geoefend worden. Deze kolonie beslaat een oppervlakte van 50 vierkante mijlen en gemiddeld zijn daar 500 kolonisten, die van een superintendent, drie Amerikaansche- en vier Filippino-assistenten het noodige onderricht ontvangen.

Behalve de reeds genoemde strafinrichtingen zijn er nog 39 kleinere gevangenissen over de Philippijnsche eilanden verspreid en een opvoedingsgesticht voor jeugdige booswichten.

En hiermede wensch ik afscheid te nemen van Manila en de Amerikanen. Hetgeen boven werd medegedeeld, had geenszins de bedoeling, door vergelijking van hetgeen op de Philippijnen door de Amerikanen werd tot stand gebracht met de toestanden van deze gewesten, kritiek uit te lokken. Een zoodanige vergelijking zou noodzakelijk op vele punten mank gaan. Ook in onze koloniën kan met rechtmatigen trots gewezen worden op vele uitmuntende inrichtingen op verschillend gebied, die ik hier niet nader behoef aan te duiden. Het zou zelfs niet moeielijk zijn er te noemen in wier schaduw gelijknamige inrichtingen op Manila niet kunnen staan. Mocht ik hier één wensch uiten, dan zou het deze zijn, dat wij van de Amerikanen een weinigje van de voortvarendheid konden overnemen, waarmede veranderingen en verbeteringen, die nu eenmaal noodzakelijk gebleken zijn, worden tot stand gebracht. Wij Hollanders sieren ons zoo gaarne met den titel van bedachtzame lieden, „die niet gaarne over ijs van één nacht gaan, de kat nog wel eens uit den boom willen kijken". Doch zoo dikwijls is die zoogenaamde bedachtzaamheid, hoe groot een deugd zij op zich zelve ook zijn moge, slechts vermomde besluiteloosheid.

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85

Haupt- und Residenzſtadt Stuttgart.

Ordnung

für die

Erhebung der ſtädtiſchen

Fleiſchabgabe.

1885.

Stuttgart.
Druck von Alfred Müller.

Stuttgart.

Bekanntmachung,

betreffend die Fleiſch-Abgabe-Ordnung.

Mit Genehmigung der K. Staats-Miniſterien des Innern und der nzen vom 31. Dezember 1884 iſt eine durchgreifende Reviſion der Ordnung Erhebung einer ſtädtiſchen Verbrauchsabgabe von Fleiſch mit weſentlichen tzen und Ergänzungen und zugleich eine Ausdehnung der Steuerpflicht auf in § 4 bezeichnete Federwild und Geflügel vorgenommen worden.

Die neue Ordnung, welche hienach veröffentlicht wird, tritt mit dem pril 1885 in Kraft.

Den 22. Januar 1885.

Gemeinderat.
Vorſtand Dr. Hack.

Fleiſch-Abgabe-Ordnung.

Für die auf Grund der Geſetze über Beſteuerungsrechte der Amtskörper- ften und Gemeinden vom 23. Juli 1877, Art. 18—25 und vom 8. März 1, Art. I. und II., ſowie auf Grund der Königlichen Verordnungen vom Oktober 1877 und 26. März 1881 eingeführte örtliche Verbrauchsabgabe Fleiſch kommen folgende Vorſchriften zur Anwendung:

Steuerpflicht.

§. 1.

Der Fleiſchſteuer unterliegen nach Maßgabe der folgenden Beſtimmungen Fleiſch und die Fleiſchwaren, welche im Stadtdirektionsbezirk zum Ver- uch kommen.

Schlachthaus-Verkehr.

§. 5.

Wer Schlachtvieh, geſchlachtete, unzerlegte Tiere, Fleiſch, Fleiſ
oder Wildbret in den Viehhof oder in die Räumlichkeiten des Schlach
einführt, hat ſolches ſofort bei der Einbringung behufs Feſtſtellung der
pflicht dem Schlachthauskontrolleur anzuzeigen.

Von Schlachtvieh, von geſchlachteten unzerlegten Tieren und von
bret iſt die Gattung und Stückzahl, von Fleiſch die Sorte und die Zah
Fleiſchſtücke, von Fleiſchwaren die Art und die Zahl der Stücke anzuge

Bei der Einbringung uneröffneter Eiſenbahnſendungen genügt die
zeigung des Frachtbriefs. Uneröffnete Poſtſendungen ſind dem Kontro
vorzuzeigen. Die Auspackung ſolcher Eiſenbahn- und Poſtſendungen ha
mittelbar vor der Wägung in Anweſenheit des Wagmeiſters oder eines
wächters zu geſchehen.

Die für die Anzeige (Abſ. 1 u. 2) ausgeſtellte Beſcheinigung (Paſſier
ſ. §. 29 der Schlachthausordnung) iſt bei der Fleiſchſchau (§§. 32 un
der Schlachthausordnung), auf Verlangen auch dem im Viehhof und Sch
hauſe anweſenden Aufſichtsperſonal und dem Steuerwächter vorzuzeigen
bei der Abwägung der ſteuerpflichtigen Gegenſtände dem Wagmeiſter
Eintrag des Wägeergebniſſes vorzulegen.

§. 6.

Wer der Fleiſchſteuer unterworfene Gegenſtände außerhalb der Betri
zeit des Schlachthauſes (§. 3 der Schlachthausordnung) in den Viehhof
in die Räumlichkeiten des Schlachthauſes einbringt, hat hiebei dem je
dienſtthuenden Wärter Anzeige nach §. 5 Abſ. 2 zu machen.

Sofort nach Eröffnung des Schlachthausbetriebs, ſpäteſtens bis Mor
um 7 Uhr, iſt von Seiten des Einbringers oder des Eigentümers der ſt
pflichtigen Gegenſtände, oder von dem Stellvertreter des Eigentümers,
gleiche Anzeige bei dem Schlachthauskontrolleur zu erſtatten. (§. 5.)

§. 7.

Vor Eröffnung des Schlachthausbetriebs (§. 3 der Schlachthausord
dürfen die eingebrachten geſchlachteten, unzerlegten Tiere weder feilge
noch verkauft, noch auf dem Verkaufsplatz im Viehhof oder in den Sch
räumen aufgehängt oder ausgelegt werden. (S. auch §. 8 Abſ. 2.)

Das eingebrachte geſchlachtete Fleiſch und die Fleiſchwaren ſind, eh
der Schau unterſtellt und feilgeboten oder verkauft werden, dem Wag
vorzuzeigen und durch denſelben abwägen zu laſſen; ehe die Wägung er

ürfen jene Gegenſtände auch nicht auf dem Verkaufsplatze im Viehhof oder en Schlachträumen aufgehängt oder ausgelegt werden.

§. 8.

Auf Grund der von dem Wagmeiſter durch Eintragung des Wäge- bniſſes ergänzten Beſcheinigung (§. 5 Abſ. 4) iſt im Verwaltungsbureau Schlachthauſes die Fleiſchabgabe nebſt der ſtädtiſchen Fleiſchſchaugebühr rt nach der Wägung und Beſchauung des Fleiſches und ehe dasſelbe aus Viehhofe oder Schlachthauſe entfernt wird, zu entrichten.

Geſchlachtetes unzerteiltes Kleinvieh, (Kälber, Schafe, Ziegen, Ziegen- ken, Lämmer, Spanferkel) und unzerlegtes Wild dürfen ungewogen aus Schlachthauſe entfernt werden, wenn die Fleiſchſchau vorgenommen worden und die Fleiſchabgabe ohne Wägung feſtgeſtellt werden konnte und nebſt Fleiſchſchaugebühr bezahlt worden iſt.

Vor dem Verlaſſen des Viehhofs oder Schlachthauſes iſt die Beſcheinigung ſſierſchein) dem Schlachthauskontrolleur zur Abſtempelung vorzulegen und Verlangen auch dem Steuerwächter vorzuzeigen.

Die ſteuerpflichtigen Gegenſtände können von dem Steuerwachperſonal zum Nachweis der Bezahlung der Fleiſchabgabe im Schlachthauſe zurück- alten werden.

§. 9.

Wer im Schlachthauſe von einem Andern eingeführtes Vieh ſchlachtet, hievon am Tage der Schlachtung dem Schlachthauskontrolleur Anzeige zu hen und die Gattung und Stückzahl der Schlachttiere anzugeben.

Im Uebrigen finden §. 5 Abſ. 4 und §. 8 Abſ. 2—4 und, ſofern die ſchabgabe nicht von dem Einbringer des Schlachtviehes entrichtet worden iſt, h §. 8 Abſ. 1 Anwendung.

§. 10.

Wer im Schlachthauſe oder Viehhof Fleiſch, Fleiſchwaren, in §. 8 ſatz 2 bezeichnetes geſchlachtetes Kleinvieh oder Wildbret kauft, hat hievon Kaufstage und vor Entfernung der ſteuerpflichtigen Gegenſtände dem Schlacht- skontrolleur Anzeige zu machen.

Die hierüber ausgefertigte Beſcheinigung (Paſſierſchein, ſiehe §. 29 der hlachthausordnung) iſt dem Wagmeiſter zur Eintragung des Namens des käufers und bei Fleiſch und Fleiſchwaren des Gewichts, bei unzerlegtem invieh und Wildbret der Stückzahl, eventuell auch des Gewichts, vorzulegen.

Im Uebrigen gilt §. 8 Abſ. 2—4.

§. 11.

Wer im Viehhof oder in den Räumlichkeiten des Schlachthauſes befind- e lebende Schlachttiere verkauft, hat hievon am Verkaufstage dem Schlacht-

hauskontrolleur Anzeige zu machen und hiebei die Namen des Verk
Käufers und die Zahl und Gattung der Schlachttiere anzugeben.

§. 8 Abſ. 3 findet ebenfalls Anwendung.

§. 12.

Wer aus dem Viehhofe oder aus den Räumlichkeiten des Schla
lebende Schlachttiere entfernt, hat bei dem Ausgange dem Schlacht
trolleur, und wenn die Ausfuhr außerhalb der Betriebszeit des Schlach
ſtattfindet (§. 3 der Schlachthausordnung), dem Stallwärter, Anzeige zu
und hiebei die Gattung und Stückzahl und den Beſtimmungsort de
zuführenden Tiere, ſowie den Namen des Einbringers anzugeben.

Fleiſcheinfuhr.

§. 13.

Wer zum Verkauf oder zum Feilhalten oder zum Verbrauch in
ſchaften beſtimmte geſchlachtete unzerlegte Tiere, Fleiſch, Fleiſchwar
Wildbret in die Stadt Stuttgart oder in die Vorſtadt Heslach einbr
hat unverzüglich, d. h. vor der Verbringung der ſteuerpflichtigen Gege
an einen andern Ort und ehe dieſelben verkauft oder feilgeboten werden,
weder im Schlachthauſe (§§. 5 und 6) oder bei dem Steuerwächter
Steuer-Eingangsſtelle, bei welcher gemäß §. 38 der Schlachthausordnung,
die Fleiſchſchau vorgenommen wird, Anzeige zu machen, die ſteuerpfl
Gegenſtände vorzuzeigen, das geſchlachtete Fleiſch und die Fleiſchwar
wägen zu laſſen und den Steuerbetrag zu entrichten.

Die hiefür ausgeſtellte Beſcheinigung iſt bei der Fleiſchſchau vorz

Wer ſolche ſteuerpflichtige Gegenſtände mittelſt der Poſt oder Ei
bahn zugeſendet erhält, iſt verbunden, der vorbezeichneten Auflage
Vorlage der mit der Sendung eingekommenen Begleitpapiere, oder, wo
nicht beigegeben waren, der Adreſſe, ſpäteſtens am Tage nach dem E
nachzukommen; vor Erfüllung dieſer Verpflichtung iſt der Verkauf un
Feilhalten der zugeſandten Gegenſtände, ſowie deren Verwendung in
ſchaften nicht geſtattet.

Die vorgezeigten Gegenſtände können von der Steuer-Erhebungs-
Kontrolleſtelle bis zur Steuerzahlung in Verwahrung genommen werden.

Sind die ſteuerpflichtigen Gegenſtände für den Haushalt des Einbri
bezw. Empfängers beſtimmt, ſo genügt eine Anzeige, die ſpäteſtens im
des nächſtfolgenden Tages beim Verbrauchsſteueramt oder bei dem Steuerw
auf einer Eingangsſtelle zu machen iſt. Anzeigepflichtig iſt der Einbr
bei Poſt- und Eiſenbahnſendungen der Empfänger.

Der Einbringer ſteuerpflichtiger Gegenſtände, der ſolche gegen Belohnung rdert (Poſt und Eiſenbahn ausgenommen), hat die Steuerpflicht ſtets nach ſchrift des Abſ. 1 zu erfüllen; es kann dies aber, wenn die Fleiſchſchau geboten iſt, (§. 38 der Schlachthausordnung) bei jeder beliebigen Steuer- angsſtelle geſchehen.

Beſondere Beſtimmungen für Berg und Gablenberg.

§. 14.

Wer in der Vorſtadt Berg oder in dem Weiler Gablenberg), deſſen Fleiſch zum Verkauf beſtimmt iſt, ſchlachten will, hat dem für Steuerkontrolle aufgeſtellten Offizianten vor der Schlachtung Anzeige zu en, die hiefür erhaltene Beſcheinigung bei der Fleiſchſchau vorzuzeigen und Abgabe ſofort nach der Schlachtung zu entrichten.

Auf die Vorſtadt Berg und den Weiler Gablenberg finden, ſoweit nicht Beſtimmung des nachfolgenden §. 15 Abſ. 1 zutrifft, die Vorſchriften in 3 mit der Maßgabe Anwendung, daß die Anzeige, bezw. das Vorzeigen, r die Entrichtung der Abgabe bei den dort aufgeſtellten Steuerbeamten nachen iſt, welche darüber Beſcheinigung auszuſtellen haben.

§. 15.

Für das in Stuttgart oder in Heslach verſteuerte und von da nach Berg Gablenberg gebrachte Fleiſch iſt eine Steuer im Ort der Einbringung zu entrichten, wenn ein nach §. 38 der Schlachthausordnung ausgeſtelltes znis der Fleiſchſchau vorgewieſen werden kann.

Dieſe Beſtimmung findet ſinngemäße Anwendung auch auf den Verkehr Fleiſch in der umgekehrten Richtung und zwiſchen Berg und Gablenberg r ſich.

Beſondere Beſtimmung für einzelne Wohnplätze.

§. 16.

Die Beſtimmungen der §§. 14 und 15 finden ſinngemäße Anwendung auf die außerhalb der geſchloſſenen Wohnbezirke gelegenen einzelnen Wohn- ze des Stadtdirektionsbezirks.

Geflügelbeſteuerung.

§. 17.

Wenn ſteuerpflichtiges (lebendiges oder totes) Geflügel auf Straßen in Stadtdirektionsbezirk eingebracht wird, an welchen ſich eine Steuerein- gsſtelle befindet, nämlich auf der

Ludwigsburgerſtraße,
Straße von Cannſtatt und von Gaisburg nach Berg,
Straße von Gaisburg nach Gablenberg und Stuttgart,
Neue Weinſteige,
Kaltenthalerſtraße,
Straße von Bothnang,

ſo iſt bei dem Steuerwächter Anzeige zu machen, die Stückzahl anzugeb Ware vorzuzeigen und abzählen zu laſſen und der Steuerbetrag zu entri

Wer hievor bezeichnetes Geflügel auf anderen Wegen oder perſönli der Eiſenbahn einbringt, hat der in Abſatz 1 gemachten Auflage unver d. h. vor der Verbringung der ſteuerpflichtigen Gegenſtände an einen Ort und ehe dieſelben verkauft oder feilgeboten werden, bei dem Steuerw einer Steuereingangsſtelle zu entſprechen.

Zerlegtes Geflügel iſt abwägen zu laſſen.

Auf die Geflügelſendungen mittelſt der Poſt und Eiſenbahn findet § Abſ. 3 Anwendung.

Die bei der Anzeige zur Beſteuerung vorgezeigten Gegenſtände von der Steuereingangsſtelle bis zur Zahlung der Abgabe zurückgehalten

Steuerpflichtiges Geflügel, das für den Haushalt des Einbringers Empfängers beſtimmt iſt, muß ſpäteſtens im Laufe des nächſtfolgenden bei dem Verbrauchsſteueramt oder bei einer Steuereingangsſtelle angezeigt Anzeigepflichtig iſt der Einbringer, bei Poſt- und Eiſenbahnſendungen der Empfä

Der Einbringer ſteuerpflichtiger Gegenſtände, der ſolche gegen Beloh befördert (Poſt und Eiſenbahn ausgenommen), hat die Steuerpflicht bei der bringung von Geflügel ſtets nach Vorſchrift des Abſ. 1 bezw. Abſ. 2 und 3 zu ent

Wer im Stadtdirektionsbezirk gezüchtetes Geflügel ſchlachtet, hat am der Schlachtung bei dem Steuerwächter einer Steuereingangsſtelle Anzeig machen und die Abgabe zu entrichten.

Schlachtung von Tieren, die dem Schlachthauszwang nicht unterli

§. 18.

Wer ſteuerpflichtige Tiere, bezüglich welcher der Schlachthausz (§. 1 der Schlachthausordnung) nicht beſteht (Ziegenkitzchen, Lämmer, S ferkel, Wild, ausgenommen Geflügel, ſ. §. 17 letzter Abſ.), zum Verkauf zur Verwendung in Wirtſchaften ſchlachten will, hat vor dem Beginn Schlachtens dem Schlachthauskontrolleur (§. 5) oder dem Steuerwächter Steuereingangsſtelle Anzeige zu machen.

Die hierüber ausgeſtellte Beſcheinigung iſt bei der Fleiſchſchau vorzuz

§. 19 Abſ. 3 und 4 finden im vorſtehenden Falle ebenfalls Anwend

Schlachtung für den Hausbrauch.

§. 19.

Wer in der Stadt Stuttgart, in ihren Vorſtädten, im Weiler Gablenberg auf den im Stadtdirektionsbezirk befindlichen einzelnen Wohnplätzen der be unterworfene Tiere (Geflügel ausgenommen, ſ. §. 17 letzter Abſ.) zu m Hausbrauch ſchlachtet, für deren Fleiſch die Beſichtigung durch die Fleiſch- nicht vorgeſchrieben iſt (Schlachthausordnung §. 1), iſt verpflichtet, vor Beginn des Schlachtens dem Steuerwächter einer Steuereingangsſtelle oder Verbrauchsſteueramt Anzeige zu machen.

Ueber die Anzeige iſt Beſcheinigung auszuſtellen.

Es iſt den Lohnmetzgern verboten, mit der Schlachtung zu beginnen, ehe ich von der Anbringung dieſer Anzeige überzeugt haben.

Die Fleiſchſteuer iſt ſofort nach der Schlachtung zu entrichten.

Schlachtung in Notfällen.

§. 20.

Wenn der Abgabe unterworfene Tiere, für deren Schlachtung die Be- ung des Schlachthauſes vorgeſchrieben iſt (§. 1 der Schlachthaus-Ordnung), dahin verbracht werden können, ſo iſt vor dem Beginn des Schlachtens einer der in §. 13 Abſ. 1 bezeichneten Stellen Anzeige zu machen.

In Notfällen, welche eine augenblickliche Tötung des Tieres erfordern, die Anzeige (§§. 14, 16, 18, 19, 20 Abſ. 1) unmittelbar nach der Tötung vor vollendeter Ausſchlachtung des Tieres zu erfolgen.

Die Fleiſchſteuer iſt ſofort nach der Schlachtung zu entrichten.

Die Lohnmetzger ſind für rechtzeitige Erſtattung der Anzeigen (Abſ. 1 2) mitverantwortlich; eine Verſäumnis derſelben unterliegt der Straf- immung des §. 29.

Hackfleiſch-Verkehr.

§. 21.

Wird Fleiſch zum Zweck des Zerkleinerns in einer Fleiſchhackerei und der herigen Wiederausfuhr eingebracht, ſo iſt hievon bei einer Steuereingangs- le Anzeige zu machen und das Fleiſch bei der Ein- und Ausfuhr abwägen laſſen. In dieſem Fall unterbleibt die Berechnung und der Anſatz der euer.

Auf das von auswärts eingebrachte, zum Zerkleinern beſtimmte Fleiſch, ches nicht wieder ausgeführt wird, finden die Beſtimmungen von §. 13 . 1, 2 und 4 bis 6 Anwendung.

Fleiſchdurchfuhr.

§. 22.

Die Durchfuhr von geſchlachteten Tieren, Fleiſch, Fleiſchwaren, bret und ſteuerpflichtigem Geflügel iſt beim Eintritt in den Stadtdi bezirk bei einer Eingangsſteuerſtelle anzumelden, die Gegenſtände zuzeigen, Fleiſch und Fleiſchwaren abwägen zu laſſen.

Bei dem Verlaſſen des Stadtdirektionsbezirks hat das Gleiche Ausgangsſteuerſtelle zu geſchehen; der Paſſierſchein iſt hiebei abzugeben.

Für Sendungen, welche der Eiſenbahn oder Poſt übergeben wer der Paſſierſchein durch die Aufgabeſtelle abſtempeln zu laſſen und hierau Steuereingangsſtelle zu übergeben.

Eiſenbahn- und Poſtſendungen ſind bei der Ausfuhr unter Vor des Frachtbriefs oder ſonſtiger Begleitpapiere bei einer Ausgangsſte vorzuzeigen und abzumelden; wenn eine Sendung geöffnet worden iſt, der Inhalt derſelben vorzuzeigen und abwägen zu laſſen.

Auf die zur Durchfuhr angemeldeten Gegenſtände, welche nicht ausgeführt werden, finden die Beſtimmungen des §. 13 bezw. der §§. 14 oder 17 Anwendung.

Fleiſchausfuhr.

§. 23.

Steuerrückvergütung wird für geſchlachtete Tiere, welche in der unzerteilt aus dem Stadtdirektionsbezirk gebracht werden, ebenſo für führtes und wieder ausgeführtes lebendes Geflügel, gewährt, ſofern die Ste entrichtung und die Ausfuhr durch Beſcheinigung nachgewieſen iſt.

Die Steuer wird im vollen Betrage, abzüglich eines auf 5 Prozent Steuerbetrages berechneten, mindeſtens aber 10 ₰ betragenden Kontrolle erſatzes zurückvergütet.

Für die Ausfuhr von einzelnen Fleiſchſtücken und von Fleiſchwaren eine Steuerrückvergütung nicht gewährt.

Abgabeverjährung.

§. 24.

Der Anſpruch auf die Nachforderung zurückgebliebener, auf die Rück gütung bezahlter und auf die Zurückforderung zuviel bezahlter Abgaben jährt in drei Jahren.

Kontrollebeſtimmungen.

§. 25.

Dem ſtädtiſchen Steueraufſichtsperſonal ſind auf Verlangen die für den der Steuererhebung ausgeſtellten Urkunden vorzuweiſen, auch ſteht demder Eintritt in das Schlachthaus zu jeder Zeit frei.

Sind Gründe zu der Vermutung vorhanden, daß ſich eine mit dem Veron Fleiſch, Fleiſchwaren, Wildbret oder Geflügel beſchäftigte Perſon einer tretung der gegenwärtigen Vorſchriften ſchuldig gemacht habe, ſo iſt dem chen Steueraufſichtsperſonal der Zutritt zu denjenigen Räumlichkeiten, zum Gewerbebetrieb benützt werden, jederzeit zu geſtatten.

§. 26.

Fleiſch, Fleiſchwaren, Wildbret und ſteuerpflichtiges Geflügel, wovon eine rentrichtung oder die vorgeſchriebene Anzeige zur Beſteuerung nicht eriſt, können von dem Kontrolleperſonal (Steuerwache, Schutzmannſchaft, hutzwache) bis zur Feſtſtellung der Steuerpflicht durch das Verbrauchsamt zurückgehalten werden.

Strafbeſtimmungen.

§. 27.

Wer der Stadtkaſſe die Abgabe von Fleiſch entzieht, wird wegen Steuerrdung um den fünffachen Betrag der gefährdeten Steuer geſtraft und hat ies die Abgabe nachträglich zu entrichten.

Die Strafe iſt verwirkt:

1. wenn in Stuttgart oder Heslach Vieh, deſſen Fleiſch für den Verkauf beſtimmt iſt, außerhalb des Schlachthauſes geſchlachtet wird (ſ. übrigens §. 20);
2. wenn beim Einbringen von Schlachtvieh, geſchlachteten Tieren, Fleiſch, Fleiſchwaren oder Wildbret in den Viehhof oder in die Räumlichkeiten des Schlachthauſes die in den §§. 5 und 6 Abſ. 1 verlangten Anzeigen nicht gemacht oder wenn hiebei bezüglich der Stückzahl unrichtige Angaben gemacht werden;
3. wenn die in §. 7 Abſ. 2 und in §§. 9, 13 14, 16 bis 19, 20 Abſ. 1 und 2 vorgeſchriebene Anzeige, bezw. Vorzeigung entweder gar nicht oder nicht rechtzeitig erfolgt, oder wenn geſchlachtete, unzerlegte Tiere, Fleiſch, Fleiſchwaren, Wildbret oder ſteuerpflichtiges Geflügel vor erfolgter Anzeige, bezw. Vorzeigung und Abwägung an einen andern Ort verbracht, verkauft oder feilgeboten

oder in Wirtſchaften verwendet werden; ferner wenn f
tiges Geflügel ohne Erfüllung der in §. 17 Abſ. 1 gem
lage auf einer der dort bezeichneten Straßen eingebracht

4. wenn zum Zweck der Zerkleinerung eingebrachtes und
angemeldetes Fleiſch in Verkaufsräumen oder in anderen
räumen als denjenigen einer Lohnfleiſchhackerei niedergel
oder wenn dasſelbe verkauft, feilgeboten oder in Wirtſcha
wendet wird, ehe es der Schau und Beſteuerung unterſ
den iſt;

5. wenn die nach §. 22 zur Durchfuhr angemeldeten Geg
anderswo, als in den Gewerberäumen eines Spediteurs
gelegt oder wenn dieſelben verkauft oder feilgeboten werden,
Beſteuerung erfolgt iſt;

6. wenn in den in §§. 5, 6, 9, 13, 14, 16, 18 bis 20
neten Fällen über die Gattung der eingeführten, bezw.
Schlachten beſtimmten oder geſchlachteten oder erlegten Ti
richtige Angaben gemacht werden.

Weist in einem der in Ziffer 1—6 bezeichneten Fälle der Angeſch
nach, daß er eine Steuergefährdung nicht habe begehen können oder
iſt anſtatt der nach Abſatz 1 verwirkten Strafe auf eine Kontrolleſtrafe
100 ℳ. zu erkennen. Auch in dieſem Falle iſt die angefallene Abgabe
träglich zu entrichten.

§. 28.

Wer eine Steuerrückvergütung erſchleicht, hat ſolche zu erſetzen u
fällt in die Strafe des fünffachen Betrags der erſchlichenen Rückvergüt

§. 29.

Alle ſonſtigen Uebertretungen der zur Sicherung der Abgabeerheb
gebenen Beſtimmungen werden mit Geldſtrafe bis zu 100 ℳ. gerügt.

§. 30.

Metzger, Viehhändler, Wildbret- und Geflügelhändler und Händl
Fleiſch und Fleiſchwaren haben für ihre Diener, Lehrlinge, Markthelfe
werbegehilfen, Ehegatten, Kinder, Geſinde und die ſonſt in ihrem Dienſt
Taglohn ſtehenden oder ſich gewöhnlich bei der Familie aufhaltenden Pe
— andere Perſonen nur für ihre Ehegatten und Kinder — rückſichtli
Geldſtrafen und Unterſuchungskoſten zu haften, welche den ſolchergeſt
vertretenden Perſonen durch Strafbeſcheid oder gerichtliches Urteil
Verletzung der Vorſchriften dieſer Abgabe-Ordnung zuerkannt worden

bei den ihnen von den Mithaftenden übertragenen oder ein für allemal
aſſenen Handels-, Gewerbs- und anderen Verrichtungen zu beobachten hatten.
Wenn die Mithaftenden nachweiſen, daß die Uebertretung ohne ihr
n verübt worden iſt, ſo haften ſie nur für die Abgabe.

§. 31.

Hinſichtlich der Zuſtändigkeit und des Verfahrens bei Zuwiderhandlungen
dieſe Ordnung wird auf die Beſtimmungen der Art. 35 und 36 des
zes vom 25. Auguſt 1879, betreffend das Verfahren der Verwaltungs-
den bei Zuwiderhandlungen gegen die Zoll- und Steuergeſetze (Reg.-Blatt
59), hingewieſen.

hang.

Auszug

aus der

Verordnung für das Schlachthaus

und den Verkehr mit Fleiſch

vom 29. Juni 1881.

§. 1.

Alles Schlachtvieh (Ochſen, Farren, Kühe, Rinder, Schweine, Kälber,
ſe und Ziegen), deſſen Fleiſch zum Verkauf oder zur Benützung in Wirt-
ſten beſtimmt iſt, muß im Stadtdirektionsbezirk Stuttgart (zur
t noch mit Ausnahme von Berg und Gablenberg) im Schlacht-
s geſchlachtet werden.

§. 3.

Die Benützung des Schlachthauſes findet ſtatt:

a) in den Monaten Mai, Juni, Juli, Auguſt von Morgens 4 Uhr, in den Monaten März, April, September, Oktober von Morgens 5 Uhr, in den Monaten November, Dezember, Januar, Februar von Morgens 6 Uhr bis Abends 8 Uhr;

b) das Schlachten an Sonn- und Feſttagen iſt nur bis Morgens um 8 Uhr, in dringenden Fällen (z. B. bei Krankheiten) jedenfalls nur außerhalb der Stunden des Gottesdienſtes geſtattet. In der übrigen Zeit bleiben die Schlachträume geſchloſſen.

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§. 29.

Zum Zwecke der nöthigen Kontrolle iſt Jedermann, der eine geſchäf Verrichtung im Schlachthauſe oder Viehhofe vorzunehmen hat, in deren Ge Gebühren zu entrichten ſind, verpflichtet, **ſofort bei ſeinem Eintritt** Schlachthaus an dem links von der Einfahrt befindlichen Portierzimmer e Paſſierſchein in Empfang zu nehmen, ſolchen dem im Schlachthauſe oder V hofe anweſenden Aufſichtsperſonal, ſowie der Fleiſchſchau vorzuzeigen, ſo auf dem Verwaltungsbureau die Gebühren zu entrichten und beim Verla des Schlachthauſes oder Viehhofes an dem oben erwähnten, bei der Ein befindlichen Portierzimmer den Schein wieder abſtempeln zu laſſen.

§. 32.

Der Schau unterliegt alles Schlachtvieh und alles Fleiſch, welches Stadtdirektionsbezirk zum Verkaufe oder zur Benützung in den W ſchaften ausgehauen wird, ſowie alle Fleiſchwaaren, welche daſelbſt zum V kaufe gebracht werden.

§. 38.

Alles von auswärts, einſchließlich Berg und Gablenberg, nach Stuttg oder Heslach zum Feilhalten oder Verkaufen gebrachte Fleiſch, desgleichen d Fleiſchwaren müſſen unmittelbar, d. h. ohne daß ſie vorher irgend ander wohin gebracht werden dürfen, auf den Viehhof oder in die ſonſt vom G meinderat beſtimmten Lokale geliefert und dort der Beſichtigung unterwor werden, wofür von der Fleiſchſchau ein die Qualität und Quantität des F ſches oder der Fleiſchwaren enthaltender Schein ausgeſtellt wird.

Iſt das Fleiſch oder die Fleiſchware mit einem gehörigen Ausweis Fleiſchſchau des Verſendungsortes (§. 37 Abſ. 2) verſehen, ſo genügt die ſtempelung desſelben oder des Paſſierſcheins (§. 29).

Bei Verbringen von in Stuttgart-Heslach geſchautem Fleiſch oder Fleiſchwaren zum Verkauf in Berg oder Gablenberg, ebenſo bei dem Fleiſ verkehr letztgenannter Orte unter ſich muß der Schein über erfolgte Beſichtig durch die Fleiſchſchau mitgeführt werden.

Fleiſch und Fleiſchwaren, welche nicht von Stuttgart-Heslach in B oder Gablenberg eingeführt werden, müſſen in den vom Gemeinderath ſtimmten Lokalen der Schau unterſtellt werden, worüber die in Abſ. 1 und vorgeſehene Beſcheinigung oder Abſtempelung zu erfolgen hat.

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DIFFERENCES IN THE HABITS OF ANOPHELINES WHICH TRANSMIT MALARIA IN AMERICA, IN EUROPE AND IN THE FAR EAST*

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Doctor Darling, whose personality and fund of experience we miss with poignant regret on such an occasion as this, used to say that to become a good malariologist one had to learn to think like a mosquito. Each malaria worker, of course, devotes himself to the psychoanalysis of the particular species with which he has to deal and constructs his program accordingly. He does not often have the opportunity to work in a number of different regions of the world, and so he thinks of malaria constantly in terms of his own particular problem. He falls into a sort of provincialism as necessary and pardonable as that of the poet who sang "What is so rare as a day in June?," forgetting for the moment that it was midwinter below the equator.

But it is now getting to be known that all anophelines everywhere do not think alike by any means, and the specialist in one mosquito must be on his guard against what Darwin called the most hazardous of human tendencies, the drawing of general conclusions from limited experience. Scientific literature now reaches an international audience and what is said here to-

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day with local conditions in mind will be read tomorrow in Italy, India, and Peking. Our soundest knowledge, then, may lead us astray if we enter unwarily into discussions with foreign workers, or recklessly publish unqualified generalizations and theories with regard to mosquito behavior and control, or, worst of all, if we reason by analogy and take the lessons we have learned through hard work or bitter experience in one region and attempt without preliminary study to apply them in another. This has been done, of course, time after time before the medical entomologist was developed and could obtain the ear of the responsible authorities. For if failure or disaster occurred in these cases, it was almost always due to the unexpected behavior of some Anopheles mosquito.

One of the most famous examples may be found in the story of the sanitation of Kuala Lumpur, capital of the Federated Malay States. After long and painful experience, rubber planters on the coastal plains had found that clearing the jungle for half a mile round about the laborers' quarters protected them from malaria, since *A. umbrosus*, the carrier, required dense shade in which to rear its larvae. The same thing, therefore, was done about the inland town of Kuala Lumpur, but with precisely the opposite effect, plunging the whole anti-malaria effort into confusion and discouragement. Malaria increased tremendously and was a heavy burden for nine years until in 1916 Strickland, an epidemiological entomologist, proved to the satisfaction of the authorities that they had to do with quite another anopheline (*A. maculatus*) of habits and inclinations diametrically opposed to those of *A. umbrosus*, preferring sunlight to shade, and running water to jungle pools. In fact, *umbrosus* was never found at all in Kuala Lumpur, and nevertheless the ranges of the two mosquitoes were not ten miles apart. "Ignorance of the distribution of *umbrosus*," says Sir Malcolm Watson,[1] "cost the country much money and many lives."

A similar entomological situation exists in certain regions of Italy, where *maculipennis,* the principal malaria carrier, will not breed in brackish water. Schemes have been evolved and in certain cases successfully carried out for letting the sea into fresh lagoons and low-lying marshes to stop anopheline breeding. The old pre-Ross belief, which held that the admixture of salt water to fresh increased the malaria, was discarded as unscientific. Recently, however, La Face[2] has revealed a hitherto unsuspected fact that in great submerged areas near the mouth of the Po, *maculipennis* is replaced by *elutus,* a mosquito very similar in appearance but different in species, an excellent malaria vector, possessing the ability to breed in salt water. Thus some basis was afforded to an ancient belief and empiricism in mosquito control brought again into well-merited discredit.

America has had such experiences in its tropical possessions. It was found in Panama that the *bromelia,* a parasitic plant growing on the limbs of trees, and holding a quantity of water in its cup-like heart, was widely distributed and was breeding anophelines. In some countries it has been considered a cause of malaria. The project was discussed of seeking out and destroying all these plants. It is probable that Darling saved the Panama Canal hundreds of thousands of dollars by proving in time that no malaria-carrying mosquito was being produced by the bromelias of Panama, and that they could be ignored.

In the Philippines our occupying troops were faced with the problem of selecting without delay a healthy location for a large permanent military station. On the basis of the best American experience and of local opinion, it was placed in the hills at a considerable distance from the coastal swamps and marshy plains. Unfortunately subsequent experience and careful surveys have shown that *A. minimus,* the principal malaria carrier in the Philippine islands, breeds only in foothill streams and nowhere on the plains, while *ludowii,* the mosquito of the coastal mangrove swamps, so dangerous in Singapore, is quite innocuous around Manila Bay. The camp had, therefore, been located in the exact center of the most malarious zone of the Island.

Such examples, however, of action based on false premises through ignorance of the differences in behavior of anophelines are drawn mainly from the past. Present day opinion on this subject is crystallized in one of the principal conclusions approved at the recent international conference of malariologists in Geneva in June of this year. Each government is urged to create a small body of trained research workers in malaria, spending all their time in scientific investigation and acting as advisers to the health authorities in the control of malaria. Collaboration between such bodies all over the world, coordinated possibly by some such agency as the Health Section of the League of Nations, would permit the organization of programs of international investigation of certain questions at present unanswerable, and might throw light on some of the theories which now seem to divide the malariological opinion of the world into something like opposing schools of belief.

I would like to illustrate the importance of the international viewpoint by referring to one or two such theories now being actively discussed.

A group of very well-known European malariologists now holds, in substance, that malaria is an anomaly in a civilized country; it is a typical concomitant of abandoned or under-developed lands and primitive conditions where poverty and ignorance chiefly characterize society. In their opinion, malaria will tend to disappear automatically in the presence of intensive cultivation and improved standards of living. Endemic malaria is thus not so much a medical or entomological problem as a social and economic one, and hence it is to the last degree futile to set about attacking the mosquito while these problems remain unsolved. The progress of civilization, aided by a liberal distribution of quinin, will cause malaria to melt away like snow, even though the anophelines remain undiminished in number.

Now, I think it can be sustained that this spontaneous disappearance of malaria in long-established and heavily-infected communities where no measures at all have been consciously taken against Anopheles, is a phenomenon to be observed only in temperate climates, and is possibly limited to Europe. Certainly it is never seen in the tropics, where nevertheless numerous areas of intensive cultivation and considerable prosperity are to be found. Here a reasonable protection from malaria is obtained only at great labor and expense, and no one would maintain for a moment that elaborate agricultural development and a high standard of living would by themselves keep malaria permanently out of the community were the Anopheles allowed to remain. Recent studies of Missiroli and myself[8] point to the fact that "anophelism without malaria" is linked up with the food habits of the European anopheline, *A. maculipennis,* which in certain regions no longer bites man. This is supported by the fact that the introduction of malaria carriers into these regions no longer gives rise to secondary cases of malaria.

Another theory recently proposed both in England and Brazil is that most of the malaria is being transmitted by a relatively few house-loving, well-protected, long-lived and lazy female anophelines, and that the great bulk of the anopheline population is practically not concerned at all in the spread of the disease. Hence house-catching of resting anophelines has been exalted to an important place in methods of malaria control, and generally recommended. Whatever the validity of this theory in regions where anophelines remain in houses after biting, it can have no reference to the major malaria problem of the Far East, where it seems almost to be a rule of etiquette for the mosquito to leave the room immediately after meals. I have myself been guilty of criticising a survey of the anophelines of Ceylon made by H. F. Carter, the Government entomologist, on the ground that the relative densities of species were based on larva counts rather than adult catches. I had no idea until I visited the Far East how difficult it is to lay hands on the adults of most of the principal malaria carriers. In Ceylon, a night's work in houses and stables and around tethered animals out-of-doors on a highly malarious cocoanut estate yielded a few *rossii* and *barbirostris*, neither of which is concerned with malaria transmission, while of *culicifacies* or *listonii*, the real malefactors, we saw not a trace. Some sort of trap must be employed, such as a defective bed net. In Malaya, *A. maculatus* disappears completely in the daytime, and though a tiny and fragile insect, subjects itself to the perils of wind and weather rather than lie up in a man-made shelter. In the Philippines attempts to inculpate *A. minimus* by dissection of specimens caught in Nature have failed through lack of material. In seven days spent in the Islands, I did not catch a single one, although a sporting colleague offered as high as a peso apiece for adult specimens. At the same time the larvae were abundant. Now, malaria is rife in all these places and it is evident the anophelines can keep it going, although if they enter houses at all they remain only long enough to bite.

I may mention also the theory that major drainage operations (referred to in Europe as great or integral bonification) have a direct effect on malaria by reducing the number of anophelines. This is an American theory, and it is undoubtedly sound, for whether the water is put underground or not, the drainage of swamps or ponds, and the putting the water into motion in canals, streams or ditches, in America is sufficient usually to abate the *quadri-*

maculatus. Even when streams are turned into ponds for power purposes, though there may be at first a period of great *quadrimaculatus* production, after four or five years in some mysterious way the waters become unattractive to the mosquito and the lake can be ignored. It seems to resume the character of a river.

Now, in Europe the *maculipennis* breeds in the edges of moving waters as well as in ponds. This is probably a temperature reaction, since it often abandons still water if it becomes too warm. But the result is that the draining of great plains and deltas, such as that of the Po and the Tiber, with the construction of ditches, intersecting channels and great collecting canals, does not greatly diminish the number of *maculipennis*. As Grassi remarked, you have merely transformed an irregular swamp into a rectilinear swamp, and mosquito production goes on as before. Yet in some cases malaria has begun to disappear after these works, whereas in others it has remained the same or actually increased on account of the increase in population which follows. Hence the European malariologist, doubtful about the relationship between the number of mosquitoes and the intensity of malaria, is driven to utter aphorisms such as "malaria flees before the plow," or (as Cavour said) that beefsteak is the best cure for malaria, or (with Marchoux) that it often seems that the intensity of malaria is in inverse proportion to the number of anophelines. Certainly a profound skepticism reigns in Europe today about the practicability of American ideas of eradicating malaria through mosquito control, and in part this has arisen from the difference in breeding habits between the principal European and the principal American anopheline.

I have cited these three theories which concern the causes of the spontaneous disappearance of malaria, the importance of the few females resting in bedrooms, and the effect of major drainage on malaria, because they are subjects of present discussion, and they seem to turn in each case on the behavior of a particular anopheline, and yet they are often discussed, refuted or defended in literature and conference as though they had general validity. Certainly international contacts and exchanges are of great importance in the science of malariology, if such it can be termed. In many cases, differences between malariologists may thus be resolved pacifically into differences between mosquitoes.

But if the anophelines which transmit malaria have such diverse habits and characteristics all over the world, it nevertheless occurs to us that there must be some profound similarity which makes of them all malaria carriers. The comparative study of them ought surely to reveal why it is that one species is a carrier and another is not. Such a generalization would be extremely useful to all malariologists everywhere.

Such comparative studies, however, bring us upon the very puzzling fact that not only do anopheline species differ one from another, but the same anopheline appears to behave differently in different situations. Take the well-known case of *A. aconitus*. Winoto (1919) found it infected in Western Java to the extent of 7 per cent, while in the same year Swellengrebel in Sumatra dissected over a thousand specimens at the height of an epidemic, and found none positive.[4] We may ask whether it is certain these were really the same mosquito varieties or strains. For example, two strains have been fairly well distinguished in the species *A. ludlowii*, one of which breeds in fresh hill streams, the other in salty mangrove swamps within reach of the highest tides. Now, *ludlowii* is another one of those anophelines which carry malaria in one region and are harmless in another. But the ability to transmit malaria is not confined to one or the other strain and is apparently not linked up at all with the known bionomic differences. The salt water variety is both the famous fever mosquito of the Javan and Malayan coasts and the perfectly harmless anopheline of the Manila fish ponds. Sections of Batavia and Singapore are rendered almost uninhabitable through the malaria caused by this insect; but in twenty years no authentic case of autocthonous malaria has been reported in Manila.

The strange case of *bifurcatus* throws some light upon the subject. In all parts of Europe it is a wild mosquito never found in houses, but in Palestine alone it enters houses freely and is a most dangerous transmitter of malaria. Is it, then, domesticity which makes carriers of mosquitoes, and can this domesticity be acquired by an anopheline in one region and not in another? The answer is, this cannot be the whole story, for *A. maculipennis* in Europe is a domestic mosquito wherever it is found, but it is not always a malaria carrier. Thus the map of malaria in Europe does not agree with the map of *maculipennis* distribution. And yet *maculipennis* is, with unimportant exceptions in a few localities, the only carrier in all this area. It is in Italy that the problem seems to be presented in its simplest terms. Why, in certain well-defined regions, is *A. maculipennis* a dangerous transmitter of malaria, and why, in other similar regions, is it perfectly harmless, even in great numbers and in the presence of gametocyte carriers? This has been the subject of numerous observations and experiments made during the past three years by Missiroli and myself at the Malaria Experiment Station in Italy. We have come to the conclusion that in that country the amount of malaria in a community is the measure of the degree of contact between *A. maculipennis* and man. This varies greatly from region to region because this anopheline, though always domestic in habits, bites both man and domestic animals, but in differing proportions in different places. Thus on the Tiber Delta one mosquito in four was taken in bedrooms (the highest rate we have yet observed, marking a considerable degree of anthropophilism); in the Pontine Marshes this "host-index" was one in nine; in Massarosa (Tuscany), where malaria appears only as a sporadic case or two at long intervals (just as, let us say, in New York State) the anophelines were plentiful, but only one in four hundred of those captured had bitten man, as shown by the precipitin reaction of the ingested blood; and in Val di Chiana, where malaria is unknown, our party was unable, in two different visits, to find a single specimen containing human blood, nor were we able to be bitten at night, although we intentionally exposed ourselves and the anophelines were very numerous. A very simple mathematical consideration will show that if only one in four hundred mosquitoes bites man, it will take the square of four hundred, or one hundred and sixty thousand mosquitoes, to make sure that the same mosquito will bite two men, a necessary condition for transmitting malaria. The probabilities are further reduced by the chances of the mosquito's dying during the two weeks which must elapse before it can become infective, and by the further chance that the first man bitten may not be a gametocyte carrier. In short, though thousands of anophelines visit the precincts of each family in Massarosa every night in summer, it might take years to fulfill the conditions necessary for a single successful transmission of malaria, even with the constant presence of a gametocyte carrier. Under such conditions, endemic malaria cannot maintain itself, and we have the phenomenon known as "anophelism without malaria." The possibility is not of course excluded that the same phenomenon, if it exists in other regions of the world, may have different causes.

What instinct or circumstance it is which induces *A. maculipennis* in certain places to bite domestic animals to the practical exclusion of man; or just what measure of contact between a given species of anopheles and man is necessary to maintain endemic malaria, cannot now be answered. But if, in spite of what I said about generalizations, I may be allowed to hazard a very conservative and obvious one, I would say that the fundamental similarity between all and diverse kinds of anophelines which transmit malaria in the world is that they all readily bite man, and bite him repeatedly, however widely they may differ in other bionomic characters.

This is so axiomatic that the field of inquiry to which it points would seem to have been insufficiently explored. No doubt it could help us to determine in the presence of a multiplicity of anopheline species the few which are dangerous to our health, and to explain why the same anopheline may be a malaria-vector in one region and quite harmless in another. But I have been interested here not so much in outlining specific problems as in trying to show that entomology in the service of epidemiology is a fundamental necessity in the study and control of malaria.

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DISCUSSION (*Abstract*)

Dr. I. J. Kligler, New York, N. Y.—It is certainly true that the great point of dissension between malariologists is due to the fact that we have limited our sphere of activity rather too closely and have been thinking more of malaria and less of the mosquito. We have assumed that the only consideration is the human, and that the mosquito is not an entity worth worrying about.

I am not familiar with conditions where one mosquito in 400 bites man; most Anopheles I am familiar with bite man too frequently for our comfort and pleasure.

James maintains that infected Anopheles stay in the houses when they become infected.

It so happens that the mosquito we have in Palestine, *A. elutus*, resembles the *maculipennis* existing in Europe and England. For the last year and a half we have examined the infected mosquitoes in houses and stables throughout the various seasons of the year and found a very peculiar condition. We found that *elutus* and *superpictus* behave very differently. Infected *elutus* live more consistently in houses; yet they change their habits: they stay in houses during June to September, but a large part of the infected mosquitoes pass out of doors during the period from September to January.

A. superpictus does not even behave during the summer as does the *A. elutus*. Some are found in houses and some in stables during the summer as well as in the winter.

Various species of *Anopheles* in different parts of the world concerned with malaria differ markedly in their habits. To base, therefore, a broad generalization of the kind which may become one of the principles of the Malaria Commission of the League of Nations on the basis of experience or experiments with one species in one part of a country where malaria is not very prevalent is, to say the least, an unjustified procedure. Such a generalization would affect harmfully the attempts at control of malaria in other parts of the world.

I might cite a single instance of the danger of generalizations from local experience. We have not had the experience of Dr. Hackett where ditching a large, flat marsh has brought about breeding of the same species of mosquito. We have found a transformation in the fauna. *Elutus* is a marsh mosquito, like the *maculipennis*. Ninety-five per cent of the Anopheles in an area were *elutus*. Then the area was drained and very shortly after that the mosquito population changed entirely: instead of 95 per cent *elutus*, the prevailing species was *A. superpictus*, which prefers running to stagnant water. The malaria problem was not solved by the drainage, but control became easier. Had we not been following the habits of the different mosquitoes, we should have found ourselves face to face with a very serious situation; as it was, we were expecting this change and waited long enough for the change to take place, and then took the necessary measures and averted a disaster or disappointment.

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THE IMPORTANCE AND USES OF PARIS GREEN (COPPER ACETO ARSENITE) AS AN « ANOPHELES » LARVICIDE.

By L. W. HACKETT

(From the Stazione Sperimentale per la Lotta Antimalarica, Roma)

FIRST INTERNATIONAL CONGRESS ON MALARIA
OCT. 4 - 6, ROME, Italy

THE IMPORTANCE AND USES OF PARIS GREEN (COPPER ACETO ARSENITE) AS AN « ANOPHELES » LARVICIDE. (*)

L. W. HACKETT, M. D., Dr. P. H.

The importance of Paris green (**), or Schweinfurt's green, as a cheap, innocuous and extraordinarily efficacious larvicide for *Anopheles* larvae, and hence as an invaluable weapon to our hand for malaria control, has not been generally recognized except by a few American field-workers. The larvicidal properties of Paris green were described by Barber (1) in 1921, and much practical experimentation during the last four years has fully confirmed the value of his discovery, but its use has not spread to Europe and it is not yet mentioned in recent standard treatises on malaria. This larvicide has been employed on a large scale by the writer and his associates in several regions of Italy during the malaria seasons of 1924 and 1925. We are convinced by this work that we now have a very practicable method of controlling the breeding of *Anopheles* mosquitoes, of universal applicability; that its cost is within the resources of the average European community and that the outlook for malaria control through anti-mosquito measures is rendered definitely more encouraging by the addition of this weapon to our armamentarium.

SUMMARY OF PREVIOUS WORK.

Roubaud (2) in 1920 successfully used trioxymethylene (paraformaldehyde) to poison anopheline larvae, dusting it on the surface of the water, where it was eaten by the larvae. The surface tension layer of

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(*) The work on which this paper is based was conducted with the support and under the auspices of the International Health Board of the Rockefeller Foundation and the Italian Health Department.

(**) The name Paris green in Europe is applied to various chemical products, including aniline derivatives without arsenic.

(1) BARBER, M. A. and HAYNE, T. B. – *Arsenic as a larvicide for Anopheline Larvae,* « U. S. Public Health Reports », Dec. 9, 1921, V. 36, p. 3027-3034.

(2) ROUBAUD, E. – *Emploi du trioxyméthylène en poudre pour la destruction des larves d'Anophèles.* « Compt. Rend. Hebd. des Séances de l'Acad. des Sc. ». June 1920, V. 170, p. 1521. *Ibid.* July 1920, V. 171, p. 51.

water will cause any fine, insoluble powder to float for some hours, whether it be heavier than water or not, and the mouth-brushes of surface feeding larvae sweep in the floating particles from a considerable distance. The larvae swallow all floating substances that are small enough to enter the mouth easily, and are quite indifferent, according to Barber (1), as to whether they are food or poison. No bait of any kind is required to make the larvae eat anything that is offered them. Barber (1), searching for a substance cheaper and more poisonous to the larvae than trioxymethylene, experimented with compounds of arsenic, and of these *copper aceto arsenite* (Paris green or Schweinfurt green) proved most efficient, the minimum lethal dose being smaller than that of any other poison tested. His field experiments showed that the powder is conveniently handled by diluting it with 100 volumes of inert dust; that it is efficient as an *Anopheles* larvicide in any type of breeding place and without previous removal of aquatic vegetation, in the proportion of 1 c. c. to 9 square meters of water surface; that it is harmless in this amount to domestic animals which may drink the water, to fish and to all forms of aquatic life including culicine larvae or other insect larvae, excepting only the surface-feeding anopheline larvae; and that its cost is so low as to put it in the same class with the poisons commonly used in large quantities by farmers to protect their crops from insect pests.

Hayne (3) and others (4) used Paris green on a large scale in 1922 with good results, low costs, and no accidents, in various regions of the United States.

In 1923, King (5) of the Bureau of Entomology, U. S. Department of Agriculture, in cooperation with the Army Air Service, began a series of interesting experiments, which are still being carried on, to test the possibilities of employing airplanes to control *Anopheles* mosquito breeding in large swamp areas and rice fields, using Paris green as a larvicide in a 5 to 10 % mixture with a fine silica earth. The method was suggested by the success of attempts to dust cotton with calcium arsenate from airplanes (6). In the Paris green experiments, dry glass slides and open pans containing *Anopheles* larvae were distributed over the area to be dusted, so that the distribution of particles of Paris green and their larvicidal efficiency could be determined. In addition results were tested by dipping for larvae in all parts of the area before and after dusting. Some of the earlier results were invalidated by the use of an inferior quality of Paris green, but in the most recent trials, *Anopheles* reduction over large areas varied from 78.5 % to 99 %.

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(3) Hayne, T. B. – *Paris green as a larvicide:* results of the season of 1922. *Trans. Fourth Ann. Conference Mal. Field Workers. Public Health Bull.* No. 137, Sept. 1923, p. 41.

(4) Discussions by Griffitts, T. H. D., Fischer, L. M., Barber, M. A., Rector, N. H. – *Trans. Fourth Ann. Conference Mal. Field Workers*, l. c., pp. 46, 47, 48, 177.

(5) King, W. V. – *Sixth Ann. Conference Malaria Field Workers.* New Orleans, 1924.

(6) *Bull.* 1204, U. S. Dept. of Agr. « Dusting Cotton by Airplanes ».

In 1923 and 1924 Paris green was put to practical service by Kligler and Shapiro (7) in Palestine; by Boyd and Davis (8) in Brazil and Tiedeman (9) in the Philippine Islands. Shapiro found Paris green one-fifth as expensive as oil and much more effective under certain conditions. Analysis of Paris green at the Government Laboratory in Jerusalem showed that it contained 2.2 percent of arsenic soluble in water. A few samples of treated water were analysed. The surface water contained amounts of arsenic in solution varying from 2.5 parts per million to 0.4 of one part per million. Water taken from the depth of the pools varied from 5 parts to 0.5 parts per million of arsenic. The surface-feeding fish were not affected by Paris green nor were culicines (which feed below the surface).

Finally Barber (10) in 1925 has tested other methods of distributing Paris green than though the air. He mixes Paris green with any inexpensive mineral oil which will spread rapidly on water. The mixture breaks up into small islands of oil which float far beyond the point of application, their distribution being aided by winds and currents. *Anopheles* larvae ingest the floating droplets of oil and the arsenic is effective although more slowly than when ingested in the form of fine dust.

The poisoning of water for other forms of life than anopheline larvae has rarely been noted. Shapiro (7) once noted dead tadpoles after the use of Paris green and Davis (8) mentions the poisoning of some ducks and chickens with Paris green in Brazil, but under what circumstances he does not say. On the other hand, all other experimenters are unanimous in stating that when used, even lavishly, for larvicidal purposes, Paris green does not kill the little top-feeding minnows or other fish, and does not harm domestic animals. Barber (4) says: « In as much as very « small amounts of Paris green are spread, the danger to persons or stock « through drinking water of treated ponds seems practically nil. Stock « pastured in the immediate vicinity of ponds repeatedly treated have « suffered no harm ».

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(7) KLIGLER, I. J. and Shapiro, J. M., *Proc. Eighth Ann. Meeting of the Anti-malarial Advisory Comm.*, Palestine, 22 Nov. 1923. M. S. from Dept. of Health. Jerusalem summarized in Sanitation Supplement No. 2, *Trop. Dis. Bull.*, June, 1924, p. 81-83.

(8) Unpublished reports by BOYD, M. F. and DAVIS, N. C. to *Int. Health Board*, N. Y.

(9) Unpublished reports by TIEDEMAN, W. D. to *Int. Health Board*, N. Y.

(10) BARBER, M. A. – *Some further experiences with Paris green as a larvicide for Anopheles larvae.* « Trans. First International Congress on Malaria ». Oct. 4-6, 1925, Rome, Italy.

EXPERIMENTS IN ITALY, 1924-25.

During the past two years the writer has carried out a number of experiments in Italy to test the efficacy of Paris green in various amounts under varying conditions of riparian and aquatic vegetation and floatage, in still and running water, and with differing densities and species of anopheline larvae. Measured quantities of Paris green mixed with 100 volumes of road-dust, were distributed over measured areas of water surface. This was done either by hand, throwing the mixture into the air so that the wind would carry the dust-cloud over the surface of the water, or by a blower like that used by Italian *contadini* to put sulphur on their vines (Fig. 1). Only localities were chosen where larvae enjoyed the maximum protection from an exuberant growth of algae and other submersed vegetation and where the proper distribution of Paris green was rendered difficult by thick marginal stands of reeds, cane and under-brush. The photographs give an idea of the conditions as regards aquatic and marginal vegetation, conditions which absolutely prohibited the use of oil as a larvicide. Not the slightest attempt was made to remove vegetation or alter the natural conditions. The density of larvae was measured by noting the average number taken in 25 dips. Protocols of 9 tests are abstracted below:

TYPE OF WATER	Water surface occupied by algae	BANKS	Area	C. C. of Paris green		Larval density		RESULTS — Larva destruction
				Total	per m²	before	after	
	%		m²					%
1. Pool	100	Clean	24	0. 5	0. 02	8. 0 [(c)]	1 in 3 dips	96
2. Pool	85	Thick reeds invading water.	80	3. 5	0. 04	5 0	1 larva found	99 +
3. Pool (*a*) (Fig. 2)	60	Heavy bush and cane.	120	6. 0	0. 05	7. 5	larvae dipped twice	98 +
4. Ditch (flowing)	30	Grassy	100	5. 0 [(b)]	0. 05	2. 0	0	100
5. Ditch (flowing)	30	Grassy	100	10. 0 [(b)]	0 10	3. 0	0	100
6. Stream (*a*) (Fig. 3) . .	10	Thick reeds and grass invading water.	200	20. 0 [(b)]	0. 10	5. 0	0	100
7. River (*a*) (Fig. 4) . . .	50	Cane, reeds, grass. . .	500	50. 0	0. 10	7. 0	0	100
8. Pool	50	Grass, reeds, wood floatage.	40	7. 0	0. 18	3. 0	0	100
9. Pool	75	Heavy reeds	400	100. 0	0. 25	7. 0	0	100

(*a*) See photo. — (*b*) Paris green distributed by blower. — (*c*) Average number taken in 25 dips.

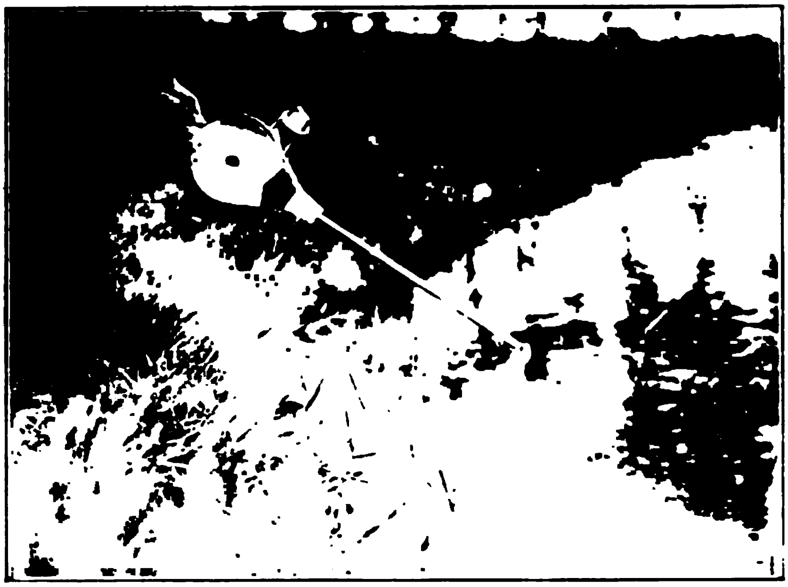


FIG. 1. — Distribution of Paris Green with hand blower.

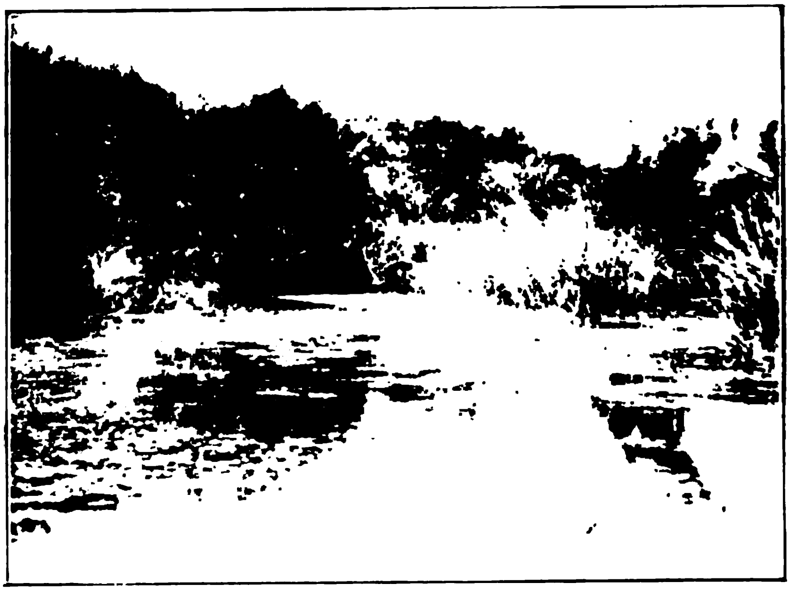
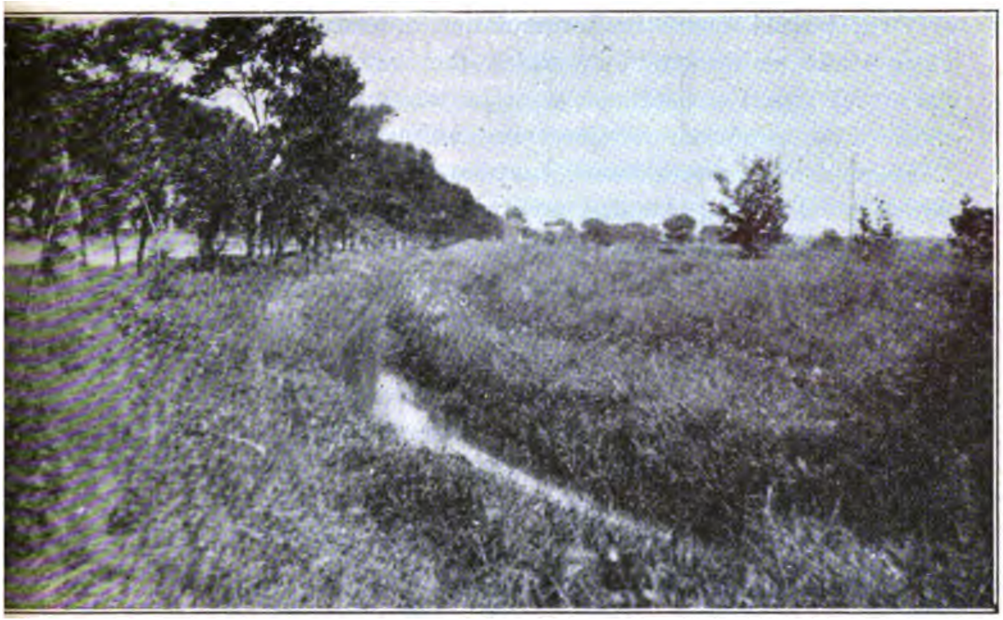


FIG. 2. — Pool at Posada (Sardegna), showing horizontal and vertical vegetation, successfully treated with Paris green.



IG. 3. — Stream at Maccarese, slow current, rich in vegetation. Complete destruction of anopheline larvae with one-tenth c.c. of Paris green per linear meter of bank.

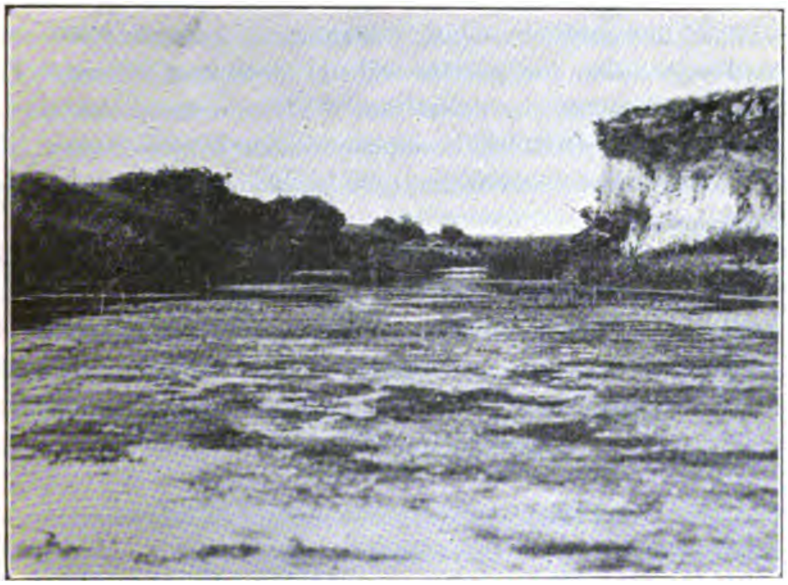


FIG. 4. — River Turitano, Portotorres (Sardegna). Complete destruction of anopheline larvae with one-tenth c.c. of Paris green per square meter, in spite of heavy horizontal vegetation.

The possibility of the accumulation of arsenic in solution in small stock-ponds or reservoirs in which the water is not replenished, but undergoes repeated treatment with Paris green for an entire season has received our attention. A simple but delicate test for arsenic in solution is afforded by a mould *Penicillium brevicaule*, which is among the most active in volatilizing arsenic if any occurs in the medium in which it grows. This action was studied by Gosio (11) and more recently by Missiroli (12). The typical garlic odor of arsenic can be noted over the mould when the medium contains one part in 100,000 of arsenic. In experiments now in course, samples taken daily from shallow pools of standing water give strongly positive tests for arsenic 24 hours after treatment with Paris green (*), but are always negative after 48 hours, and until the next treatment. Dissolved arsenic does not seem to accumulate therefore in such collections of water, but is rapidly eliminated, perhaps through volatilization by the microflora of the water.

The results may be summarized as follows:

(1) *Larval destruction.* — In the proportion of 1 c. c. to 10 square meters of water surface, Paris green killed all *Anopheles* larvae regardless of the amount or density of algae or marginal vegetation, and whether the breeding was in standing water or in proximity to running water, as in ditches, streams and rivers. Wherever, in flowing water larvae can maintain themselves by taking advantage of quiet bays or partially submersed vegetation, the powder will also remain a sufficient time to poison them. Even in the proportion of 1 c. c. to 20 square meters, Paris green caused practically complete destruction of larvae. In the laboratory, Paris green should never be handled in the same room with cultures of larvae.

(2) *Injury to other forms of life.* — In none of the tests (including no. 9, where 2 ½ times the lethal dose for anopheline larvae was employed), were frogs, leeches, or top-feeding fish destroyed or were culicine larvae killed. It appears that dissolved arsenic does not tend to accumulate in standing water periodically treated. In very shallow pools or containers, culicine larvae die after feeding on arsenic which has sunk to the bottom.

(11) Gosio, B. – *Studi sulle biorcazioni dell'arsenico, tellurio e selenio.* Min. dell'Interno, Dir. Gen. della Sanità Pubblica, Lab. di Batt. e Micrografia, Rome, 1906.

(12) Missiroli, A. – *Nuove ricerche sulla volatilizzazione fermentativa dell'arsenico.* « Bollett. della R. Accad. Medica di Roma », Anno 43, Fasc. V, 1917.

(*) This would indicate a more concentrated solution than that determined by Shapiro (7) by chemical analysis.

(3) *Cost.* — We estimate that a man can prepare the Paris green mixture and distribute it over 2000 square meters of water per day, at least. At a maximum daily wage of 20 lire, and an expenditure of 0.40 lire for Paris green (20 grams to 2000 square meters), the expense of treating one thousand square meters of water surface or one thousand linear meters of bank would be 10.20 lire. It is evidently labor rather than material which determines the cost of the work and this will vary from region to region.

(4) *Disadvantages.* — Paris green has no effect on eggs or pupae of *Anopheles* or on any of the aquatic stages of culicine mosquitoes. It seems probable also that anopheline larvae do not run much risk of ingesting Paris green during the first day of life.

ANTI-ANOPHELINE CAMPAIGNS IN ITALY, 1925.

The results of the above tests were so encouraging that Paris green became the chief weapon employed in anti-anopheline campaigns undertaken during 1925 in two different regions of Italy, as part of a cooperative project between the International Health Board of the Rockefeller Foundation and the Italian Health Department.

1. — Portotorres, a very malarious town of northern Sardinia, with a population of about 6000, is invaded in great numbers each year by *Anopheles* which breed in the river Turitano, a relatively tortuous and sluggish stream (see Fig. 4), which for six kilometers of its course lies within flight distance of the town. During April, May and June, the flight distance of *A. maculipennis* was underestimated at 2 kilometers, and in June *Anopheles* began to appear in stables and houses in the periphery of the town. The greatest number taken in one day in all the stations was 480 on June 23rd. The periodic distribution of Paris green was immediately extended to 3 kilometers from the edge of the town (taking in 6 kilometers of river), with the result that the average daily catch in the stations was reduced to less than 4. In August of the preceding year, the daily catch was always over 1000 *maculipennis*. Throughout the entire season, careful dipping of the river fortyeight hours after treatment with Paris green almost never revealed larvae larger than the first stage. Exceptions were unimportant and easily remedied, and on two occasions only, during the entire season were any pupae found. Up to September 15th, 243,000 sq. meters of water were reported as having been treated

with the use of 72 litres of Paris green, and 1083 days of labor used in filling, cleaning and dusting of breeding places, at a total cost of 15,270 lire (1,730 lire for larvicide and 13,540 lire for labor). The material and labor employed in the successful control of *Anopheles* mosquitoes at Portotorres, cost therefore about 2.55 lire per capita of population, which figured in United States currency amounts to about 11 cents.

2. — Bianconovo is a highly malarious town of 2,500 population situated in Calabria. There are six torrents within two kilometers of town, which though not perennial streams, breed anophelines in pools along their beds throughout the dry season. There are also two large rivers at 3 and 4 kilometers respectively from town, with adjacent marshy land which was also treated with Paris green beginning in July, since many families from the village persisted in sleeping in their fields near the river instead of returning home at night. Anopheline mosquitoes were kept at zero in all the catching stations in town throughout the season except for a brief period at the end of the season when the drying up of intervening water brought a few *superpictus* from regions beyond the present radius of operations to the outermost houses of a salient of the town toward the hills. Up to September 15th, 17,800 sq. meters were reported as having been treated with the use of 17.5 litres of Paris green, and 313 days of labor spent in filling, cleaning and dusting breeding places, at a total cost of 4,327 lire (415 lire for larvicide and 3912 lire for labor). The material and labor employed in the successful control of *Anopheles* mosquitoes at Bianconovo, cost therefore about 1.75 lire per capita of population which figured in dollars amounts to about 7 cents.

These sums, it should be mentioned, represent only a part of the total amount expended during the year at the two Malaria Experiment Stations in Portotorres and Bianconovo. The continuous scientific control of the experiments by malariologists and trained technical assistants was much more expensive than the actual work of preventing mosquito breeding. On the other hand, 50 % of the cleaning of banks and removal of vegetation done in the first part of the season was later shown to have been quite unnecessary. The expenditures recorded above enable us at any rate to estimate the probable cost of maintenance of mosquito control in these localities in future years. The amount of Paris green used was undoubtedly excessive in both stations, as was the labor employed in cutting and removing vegetation. The time actually spent in distributing Paris green was 107 working days in Portotorres and 86 in Bianconovo. One man employed for the entire year at a salary of 6000 lire, and using not more than 500 lire worth of Paris green, would be able to keep the *Anopheles* under control in Bianconovo. The total expenses would not exceed 7000 lire, or approximately 300 dollars, a per capita cost of 2.8 lire or about 12 cents, U. S. currency. In Portotorres, the man would need a helper for eight months in the year at a cost of 3200 lire, and the Paris green would cost 1300 lire. The total expenses might come to 12,000 lire or approximately 500 dollars, being a per capita cost of 2 lire, or less than 10 cents. Inspection should be at the expense of the State or Provincial Health Department.

Certain points are worth emphasizing in the consideration of this work:

(1) *Personnel.* — Highly trained employees are not needed for this work. Every Italian *contadino* understands the use of the hand-blower for the application of poison to vegetation. Trustworthy, methodical men, intelligent enough to dip for and recognize *Anopheles* larvae are to be found in every village.

(2) *Stock-pond.* — The long dry season in Calabria makes water precious, and the people will not allow the use of larvicides which render water unfit for animals to drink or women to wash clothes in. Small collections of water were treated over and over again with Paris green without causing the least harm to hundreds of domestic animals which drank there daily.

(3) *Wells.* — If wells or cisterns are a source of *Anopheles*, it is preferable to use top-feeding fish (*Gambusia affinis*), but Paris green in the proportion of one gram to 20 square meters, carefully measured, is quite safe.

(4) *Extensive submerged areas.* — In neither area did we have to deal with great swamps or large extensions of marshy plain. We successfully treated a number of small marshes in river valleys, but there is evidently a limit to the economic use of larvicides, and here the engineers must take up the problem. We have been concerned with only the so-called minor measures in the control of malaria. Wherever, however, water in submerged areas can be concentrated and systematized by drainage operations, breeding can be dominated with Paris green. And under certain conditions, as King (5) has shown, where airplanes are in use for distributing arsenical dusts in the control of insect pests of cotton or other crops, they may be economically employed at the same time to control *Anopheles* breeding over large areas of swamp.

(5) *Advantages.* — The manifest advantages of Paris green are its cheapness and portability, its uselessness for other purposes and the conse-

quent lack of temptation to thieves; its effectiveness in spite of all types of vegetation; its harmlessness to every form of life but anopheline larvae; the fact that it does not render water unfit for domestic uses; and the simplicity of its application as a larvicide.

It is not too much to say that the discovery of Paris green as a larvicide is the most important addition to our knowledge of malaria control in a decade, and that it opens up new and great possibilities in malaria prevention through Anopheles reduction.

PARIS GREEN OR SCHWEINFURT GREEN: COMPOSITION AND SPECIFICATIONS.

The chemical known as *Paris green* in the United States, and as *Schweinfurt green* in Europe, is copper aceto arsenite [$3\ Cu\ HAs\ O_3 - Cu\ (C_2\ H_3\ O_2)\ 2$] a double salt of copper arsenite and copper acetate. It contains. in the pure state, 58.62 % arsenious anhydride, and under United States law (Federal Insecticide Act of 1910), the name Paris green is restricted to products containing a minimum of 50 % arsenious oxide. The commercial product contains varying quantities of extraneous substances, but a good quality will average 55 % of arsenious oxide. It is a microcrystalline powder of emerald green color, practically insoluble in water, but almost completely soluble in ammonia, and concentrated acids. It costs 19 lire a kilogram in Rome. It should pass a 200-mesh sieve.

Paris green is so often adulterated and is known by such a variety of names that careful specification of its arsenite content should be made when ordering it for larvicidal purposes. Great disappointment has attended the use of inferior or adulterated products, as reported by Boyd (8), King (5) and in a personal communication by Grassi to the writer. Both a chemical analysis and a field test should be made on each new lot of Paris green before employing it on a large scale.

TECHNIQUE OF USING PARIS GREEN.

1. *Dilution.* — Road dust is the cheapest and best diluent for Paris green. It should be screened and stored under cover in large quantities, since only a very dry dust can be used. If sufficient amounts cannot be obtained, we have found wood-ashes, spoiled flour, lime, fine sand and

cork dust possible substitutes. Barber has recently suggested the application of Paris green mixed with oil, since larvae will ingest droplets of oil on the surface of water.

2. *Mixing.* — Failures are due to improper mixing as well as to inferior quality of Paris green. Mixing should always be done mechanically in a tight box or small barrel through which an iron pipe has been run diagonally for an axis and which can be revolved like a concrete mixer. A box 30 × 30 × 50 cms. will serve for ten litres at a time.

3. *Distribution.* — This can be done by hand, throwing the mixture in the air in such a way that the wind will carry the dust cloud over the face of the water. This is the method of choice for large water areas. It is far more satisfactory, with small bodies of water, to use the hand-blower or bellows (see Fig. 1) employed by farmers to dust vines. The blower gives a good cloud and an even spread; the dust can be directed downwards so as to reach the water even in a strong wind; it automatically measures the amount of dust applied, thus cutting down waste (substitution of blowers for hand distribution at Portotorres cut down the consumption of Paris green 50 %); in dealing with overgrown ditches and thick reeds or bushes at the water edge, the nozzle of the blower can be thrust through the encumbering vegetation and the dust delivered directly on the water instead of on the foliage of the plants; and no special training in its use is necessary for youths in agricultural communities.

4. *Quantity.* — The tendency always is to use too much. This is corrected by demonstration, by the use of mechanical blowers for distribution and by restricting the amount of Paris green in the mixture to one percent. The object is larval destruction, and it seems impossible to apply even a one percent mixture so lightly as to fail to secure this result. An exception is water covered with scum which sometimes requires considerably larger amounts. A stronger mixture than one percent might be used in case diluent material were very scarce, or had to be transported long distances at great expense. We have never had to use more than 0.1 c. c. of Paris green to the square meter, under any conditions of obstructing or protective vegetation. Thus a litre (1250 grams) of Paris green should control breeding over 10,000 square meters of surface or along 10 kilometers of bank. The intervals between treatments should be shorter than the period required for *Anopheles* pupae to develop, in that season and for that species.

5. *Precautions.* — The persons who distribute Paris green suffer no ill-effects if they take ordinary care to keep to windward of the dust-cloud,

change their outer clothing at the end of work, and wash their hands before eating. We had an occasional complaint of headache and diarrhoea in the first few days of our work in the period of hand distribution, but none in recent months. No skin manifestations nor burns were reported to us.

With regard to the water treated, it has been established that Paris green in quantities destructive to anopheline larvae does not kill culicine larvae except in shallow pools; and these, if present, may be used as a rough index of safety. Paris green does not harm fish of any kind (including *Gambusia affinis*), or domestic animals or vegetation.

SUMMARY.

1. — Paris green is the American name for copper aceto arsenite, known in Europe as Schweinfurt's green or arsenical green.

2. — The importance of Paris green as an anopheline larvicide has not been recognized except by a few American field-workers; it is not mentioned in recent standard European treatises on malaria (*).

3. — The advantages of Paris green are its cheapness and portability, its effectiveness as an anopheline larvicide on water-surface covered with algae and obstructed with thick vertical vegetation; its harmlessness to other forms of aquatic life and to domestic animals; the fact that it does not render water unfit for domestic uses; the simplicity of its application as a larvicide.

4. — The disadvantages of Paris green are that it does not affect eggs or pupae of *Anopheles* or any of the aquatic stages of culicine mosquitoes.

5. — Carefully controlled experiments in Italy showed that in the proportion of 1 c. c. to 10 square meters of water surface, Paris green invariably caused 100 % destruction of *Anopheles* larvae regardless of the amount or density of algae or marginal vegetation, and of whether the water was standing or flowing. This amount was innocuous to all other forms of life.

(*) On the basis of work described in this paper, the Italian Government has recently introduced directions for the use of Paris green in the « Technical Instructions for Mosquito Control in Antimalaria Work ». (Min. dell'Int., Decreto 28 febbr. 1925).

6. — The cost of applying Paris green in Italy does not exceed 10.20 lire per 1000 square meters of water surface. Successful anti-anopheline campaigns based on the use of Paris green in two towns in Italy in 1925 cost respectively 15,270 lire (625 dollars) and 4,327 lire (180 dollars), representing a per capita cost of 2.55 lire (11 cents) and 1.75 lire (7 cents), respectively.

7. — The composition of Paris green and specifications for ordering it are given, with a warning against the frequent adulterations of the commercial product. In Europe substances may be sold as Paris green containing no arsenic whatever.

8. — The technique of the preparation and application of the larvicide is given in detail, with the precautions to be observed.

SQUARCI – ROME

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THE LABORATORY FOR MOSQUITO RESEARCH IN ALBANIA

by **L. W. Hackett** and **Marston Bates,**
of the Field Straff of the International Health
Division of The Rockefeller Foundation

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The modern trend of malaria control is toward ,,species sanitation'' and ,,naturalistic measures'' which call for careful study of the natural history and physiology of mosquitoes of the particular region in which the control program is being carried out. The aim is to limit the attack when possible to the vector species (ignoring the relatively harmless anophelines present), and by acquiring a working knowledge of its local habits, to determine the point at which this attack can most effectively be delivered. We are particularly interested in knowing where and when these dangerous anophelines feed and upon what range of animals ; where they retreat in the daytime ; under what conditions they mate ; the qualities of the surface waters on which they lay their eggs ; and the limits of tolerance of their larvae to changing physical and chemical factors in these waters. This is a program requiring time. It cannot be carried out by a field expedition, and yet it involves a constant interaction of field observation with laboratory research which is not possible to a central institution. The study of behavior under natural, semi-natural and laboratory conditions necessitates locating the laboratory at the source of material. Selfevident as this may seem, there are very few laboratories of this kind functioning at present in malarial regions, and the object of this note is to describe in a general way the setup of the laboratory for mosquito research in Albania, and to indicate the field of biological research accessible to a small, modestly equipped station like this, in primitive surroundings, with native assistants.

The laboratory in Tirana (Albania) (Fig. 1) was not originally planned for such research, but started as a table, a sink and a microscope for the examination of blood preparations and the classification and dissection of mosquitoes, ordinary procedures indispensable to a program of malaria control. This program was soon found to embrace *sacharovi*-borne malaria along the coast, *superpictus*-borne malaria in the hills, and a *maculipennis*-borne malaria around the large inland lakes and marshes. The ecological field studies which were now begun revealed the occurrence of at least ten anopheline populations in Albania: *algeriensis*; *claviger*; *hyrcanus* (*pseudopictus*); *maculipennis* (*messeae*, *subalpinus* and *typicus*); *marteri*; *plumbeus*; *sacharovi* and *superpictus*. A full-time biologist took charge of the laboratory, which gradually acquired the structures and equipment, in large part simple and homemade, which we are about to describe.

The first addition to the laboratory proper was a very large cage built of wire screening supported on telegraph poles, in which anophelines could be liberated and their activities kept under close observation (Fig. 2). The enclosed space is 10 meters long, 5 meters wide and 6 meters high, big enough to contain a number of structures which can be built and taken down at will. The initial experiment called for a miniature Albanian farm in which, under the net, were grouped a small one-roomed house, a garden with a tree, a pool of water, a calf stable and some rabbit hutches. Later a subterranean gallery was added, which in summer could be kept humid, and in winter heated. In such a setup, several different species of anophelines can be introduced simultaneously, and something learned about the conditions which stimulate males to swarm and females to bite; daytime resting places and the proportion of mosquitoes which remain indoors; about the length of life under such conditions, and the influence of meteorological factors (temperature, wind, rain, barometric pressure, electrical disturbances, etc.) on behavior.

Next summer the scene will be changed completely. A small brick building consisting of three identical rooms placed side by side has been constructed for host-preference experiments with man and other animals. At the other end there will be several shallow concrete pools in which different combinations of aquatic plants can be assembled to test their possible influence on the egg-laying instinct. By putting the aquatic plants in shallow terra cotta flowerpots which are placed in the cement lined pools, the character of the vegetation in a particular pool can be changed at will. The behavior of ovipositing females, the time of egg-laying, and the effect on choice of breeding place of changes in the physical and chemical character of the water, can, it is hoped, be studied in this way. There is also the possibility of studying the effectiveness of natural enemies, in the water and in the air, on such an insect population.

The Big Cage was very useful for observations on behavior, but it proved to be a failure as a method of keeping self-perpetuating colonies of captive mosquitoes. The populations did not maintain themselves, whether because of excessive mortality or restricted water surface, and had to be continually restocked with material raised in the laboratory. Observations on insects admitted to the small one-roomed house inside the Big Cage showed that with the windows curtained, and under certain conditions of artificial illumination, several anopheline species would swarm, and mating would take place. A windowless insectary was therefore built for experiments with artificial illumination. It was constructed of hollow tile for insulation, and had three small rooms, one for mosquitoes in cages, and two of the same size as that in the Big Cage (2 x 4 meters, 2.5 meters high), in which mosquitoes could be liberated. Experiments with various colors, rhythms and intensities of illumination finally produced conditions under which both *sacharovi* and *superpictus* would reproduce readily. Not infrequently fertile eggs of *typicus* were found, although the males were never observed dancing and there were not enough eggs to maintain a colony. Such rooms are adapted to certain experiments and observations not possible in the Big Cage and are still big enough to permit the introduction of larger animals as a food supply, an important factor in dealing with certain species and varieties which are not easily persuaded to feed on man or such small animals as guinea pigs, rabbits and chickens. The disadvantages of room-sized cages are that they occupy too much space and there are never enough of them for the number of colonies it is desired to maintain; furthermore, finding the mosquitoes and their eggs is time-consuming and apt not to be complete.

Attempts were now made to confine colonies in even smaller spaces — in cages 50 cms. square by a meter in height. The importance of proper lighting was evident from the insectary experiments. The light conditions were varied in the small cages in a purely empirical way until some method worked, although the underlying causes were not well understood. A diffuse blue-green light of low intensity stimulated swarming, an effect not to be secured by putting the cage out of doors after sundown, which merely excited the insects to beat against the glass in an effort to escape. In a dark room with only the inside of the cage illuminated, the mosquitoes abandon their constant exploratory attempts to escape and resume the instinctive activities provoked by the conditions artificially produced in the restricted environment. The temperature should be kept around 25° C., and a relative humidity above 80% seems always to be favorable. In this way colonies of *superpictus* and the Italian *labranchiae* were easily established and have now maintained themselves for over two years. The same technique has proved successful with *A. pharoensis* and *A. multicolor* in Egypt. The cage is furnished with a small Edney paper hygrometer the size of a watch ; with a black bowl half full of water for egg-laying, with a built-in cage of chicken wire in the angle between the floor and one side in which a rabbit or chicken can be left at night, and with some sugar solution or fruit for the males to feed on. A wet turkish towel can be hung at the back if the humidity tends to fall on hot days in summer. On the top of the cage, which is of glass or ,,vimlite'' (wire screening filled with a cellophane-like material), rests the lighting arrangement consisting of a small bulb in a metal shade which stands upon a colored glass filter and produces a greenish-blue light of from 1 to 5 foot-candles. Such a cage is shown in Fig. 3.

Throughout the artificial day of twelve hours the anophelines are rendered immobile by a central lamp bulb creating a light intensity of more than 50 candles in all parts of the room. This is succeeded by an hour or so of complete darkness, after which the blue-green illumination is produced within the cage for three or four hours. The lights may be conveniently manipulated by a time switch run by clockwork. This rhythm and color effect, as we have said, were arrived at by a method of trial and error, and for other anopheline species elsewhere some different scheme may prove more successful.

The ability to maintain colonies of many different anophelines, including all the vector forms of Europe, made it possible to keep alive material obtained from other regions for study and comparison. In the beginning the laboratory was principally engaged in a study of the characters, physiological rather than morphological, which distinguish the seven or eight members of the *maculipennis* group. One valuable method of differentiation is hybridization, for which purpose the *atroparvus* male (a facultative swarmer) has hitherto been invariably used, since it is the only *maculipennis* form which will inseminate females of related varieties in cages of any size without a nuptial flight. The way is now open however to employ other males, and we have used *labranchiae*, an Italian form, with success in hybridization experiments, the males swarming readily in the small cage. The establishment of colonies as a source of material makes laboratory studies practicable at all seasons of the year ; and while sex behavior, the sterility of hybrids, and many other biological phenomena may seem to have little direct relation to malaria control, such studies bring out the true value of what often seem to be unimportant somatic characters. Morphological differences are important when they are correlated with physiological and behavioral differences, and these can only be tested out by physiological studies in the laboratory. There are many other anopheline complexes besides *maculipennis* which still await the sort of analysis to which the latter is beginning to be subjected. The field is almost limitless, and almost unexplored.

An important corollary to the differential study of nearly related forms is the bringing together in one place of living representatives of the same form from widely separated parts of its range. For example, our laboratory for some months maintained in separate colonies *atroparvus* from England, Germany, Hungary, Portugal and Italy. The comparison of these populations is important because this anopheline is a vector of malaria in some places and not in others. It is the cause of practically all the malaria of England, Holland, Germany and Portugal, but it seems to be relatively harmless in France, Hungary and Italy. Furthermore, it has already been tentatively divided by some authors into subvarieties on the basis of small morphological and physiological characters, and it is of the greatest interest to determine whether these are regional modifications or true genotypic variations.

There is also the problem presented by the anopheline species and varieties which have not yet been domesticated in captive colonies. In the *maculipennis* group, *typicus* will mate regularly and lay fertile eggs only in the Big Cage ; *sacharovi* swarms in an insectary cubicle, but not in any smaller space ; *messeae*, *melanoon* and *subalpinus* have never mated, to our knowledge, in any situation we have been able to contrive. Studies of the last three types depend on wild-caught individuals and on the resulting eggs, larvae and virgin adults bred out in the laboratory. *Melanoon* does not occur in Albania, so that we have been unable effectively to attack one of the pressing problems of *maculipennis* classification, namely the relationship of *melanoon* to *subalpinus*. Similarly in our Egyptian studies it has been possible to maintain captive colonies of *A. pharoensis* and *A. multicolor*, but not of the other two common anophelines, *A. sergenti* and *A. coustani*. It is not unlikely, however, that success in such cases is only a question of discovering the right technique.

The little insectary building, in which the colonies were kept, responded too rapidly to changes in temperature out of doors, and behavioral studies on adult mosquitoes required a room in which not only lighting but also temperature and humidity could be kept under control within narrow limits all the year round. We took advantage of the construction of a small building for experimental malaria in animals, digging a subterranean room beneath it, 2 meters wide, 4 meters long, and 2 meters high (Fig. 3). It is heated by an ordinary blue-flame oil stove which is placed in a galvanized iron box connected by a long and almost horizontal stove pipe with the outside, to avoid having smells from the stove in the room. Humidity is provided by wet towels and by sprinkling the floor, and the lights are turned on and off to make an artificial day and night by an inexpensive timeswitch run by clockwork. There are no automatic thermostats or humidity controls, but by checking conditions in the room three or four times a day, we have found that the temperature can be kept constant within one degree in the range that we have used (20° to 25° C.).

The necessity of having a large supply of newly emerged adults every day for maintaining the population of the Big Cage, for the regular colonies, and for any special experiments, led to a search for a routine method of raising larvae that could be depended upon to produce large numbers of healthy mosquitoes. Our present method consists in keeping the larvae in rainwater over a substrate of fine sedimented silt, and sprinkling dried bread crumbs over the surface of the water for food. Egg batches from the mosquito colonies are kept in rain water in an incubator at 27° C. (to insure uniform hatching) and the first-stage larvae are transferred to small enamelware bowls about 12 cm. in diameter, holding about 200 cc. of water; approximately 200 larvae are put in such a bowl. As they grow, the larvae are transferred twice into larger pans, until at fourth stage they are kept in milkpans 30 cm. in diameter, from which the pupae are collected daily. The larvae are kept in a room with a transparent roof (vimlite) admitting direct sunlight part of the day; in the winter the room is heated with a wood stove.

We have found dried bread crumbs to be a very satisfactory larva food; ordinary white bread is bought from a nearby baker, dried in the sun or on a stove, ground fairly fine in a coffee mill, and a small amount spread daily over the water surface of the larva pans. We made many experiments with such larva foods as yeast, hay infusions, algal cultures, dried blood serum, dog biscuit, but we found none of these materials to be as dependable as bread crumbs. The addition of mud to the larva pans is probably necessary as a source of mineral salts ; but the mud particles may also have some mechanical function, since an ,,extract of mud'' made by filtering a mud suspension does not seem to have the same effect as the mud itself. We use a fine sandy loam in the Tirana laboratory ; but the exact soil type does not seem to be important, since we had no difficulty in finding suitable mud in Rome and Egypt. The field soil is thoroughly mixed with rain water in the laboratory and the resulting mud is passed several times through wire gauze of moderately fine mesh to remove stones, roots and possible predatory animals ; the resulting suspension is added to the larva pans, sedimenting to form a layer on the bottom about half a centimeter deep. We use rain water for all routine operations in the Tirana laboratory because we have found that the local tap water makes a very unfavorable medium for larval growth ; in Rome also rain water seems to be preferable to water from the city supply.

,,This mud-rainwater-breadcrumb method of raising larvae'' has enabled us to breed as many as three thousand anophelines a day with the labor of one man when we have wanted such numbers for our experiments, and to raise from four hundred to a thousand adults every day throughout the year for the maintenance of our mosquito colonies. All of the species that we have tried (including such diverse forms as *maculipennis*, *superpictus* and *claviger*) seem to respond equally well to this treatment. The method is not, however, suitable for experimental work since the mud, the rain water and the bread crumbs are all of unknown composition, and liable to vary from place to place. We have made several thousand experiments with various solutions based on the use of distilled water and standard chemicals, in the hope of finding some objective technique so that experiments made in one laboratory or with one mosquito species could be compared with experiments in other laboratories or with other species. We have learned a great deal about the behavior of anopheline larvae in the course of these experiments, but we have not yet found our standard technique.

In making such larval experiments, we find a large water bath of the type designed by Boyd to be indispensable (Fig. 4). Such baths can be made and operated quite cheaply, and they make possible a very satisfactory control of temperature. The water bath shown in the photograph is one meter long, 65 cm. wide and 15 cm. deep, made of copper, insulated with compressed cork and incased in wood; we have made other baths, however, which seem to be just as satisfactory, using zinc and sawdust. The water is heated by immersed resistance wires, with thermostatic control. For work in which exact temperature control is not necessary, we made a similar bath with a small oil lamp on a shelf underneath, which heats a small circular portion of the metal bottom through an opening left in the insulation. For laboratories without electricity a thermostatic control for such a bath could no doubt be devised, using the damper system employed on oil-heated chicken incubators.

We have tried always to keep the laboratory work closely correlated with studies in the field; and in this we have been fortunate in finding a large farm near the main road and about fifteen minutes by car from the laboratory, where the owner allows us to try any experiments that we want in the stables, houses and breeding places. A small stream on this farm has been turned over to us, and using this water we have been able to make breeding places of our own devising to see what kind of mosquitoes lay eggs there, and how the larvae develop. By collecting in the stables of this farm we get a steady supply of adult material of some of the species (especially *typicus* and *subalpinus*) that we cannot maintain as colonies in the laboratory.

In general in the field work we have emphasized the study of natural breeding places. This has been confined mainly to the physical and chemical factors, and important fields of microbiology and ecology have not been touched as yet. The measurement of larval densities and mosquito production are, however, essential, and a very simple method has been devised for this, applicable to many situations. The top and bottom are cut out of an empty kerosene tin and it is thrust down quickly into the mud at the bottom of a marsh, flooded ricefield or other shallow breeding place. All the water is dipped out into a pail, algae and other floating vegetation are washed and discarded, and the larvae are then picked from the pail and counted, the number in the various stages being recorded. A galvanized iron cylinder, 36 cms. in diameter (one-tenth of a square meter in cross section) is now being used instead of the oil-tin. The relative number of larvae of each stage under ordinary conditions of mortality can be determined and variations from this formula often give valuable information about seasonal increase or diminution in the amount of egg-laying, the appearance of predators, the effect of new conditions produced naturally or artificially, etc.

The field work often determines the trend of the laboratory work ; its fundamental object is to observe the behavior of anophelines in nature and to attempt to define the conditions which determine or influence this behavior. This has led, for example, to work on traps, and on the devising of artificial shelters which anophelines might choose as daytime resting places, and many other studies of particular value in regions where anophelines do not remain in houses or stables during the day. Photographic records are frequently made in field and laboratory, and, wherever possible, physical conditions are measured by recording instruments. But a detailed description of field activities lies outside the scope of this article.

Our object has been to show the value of a biological experiment station located in the heart of a malarious region, and within easy driving distance of extensive breeding places of numerous anopheline species. It is feasible to equip and run a laboratory of this kind, at moderate cost, in a primitive place, in order to tap a rich experimental and observational field still largely undeveloped. Library facilities are lacking; but much help can be got from a single well-chosen shelf of books, a few journals, a steady flow of reprints and complete sets of the *Review of Applied Entomology* and the *Tropical Diseases Bulletin*. Annual trips to the nearest large scientific center permit the searching of a more extensive literature.

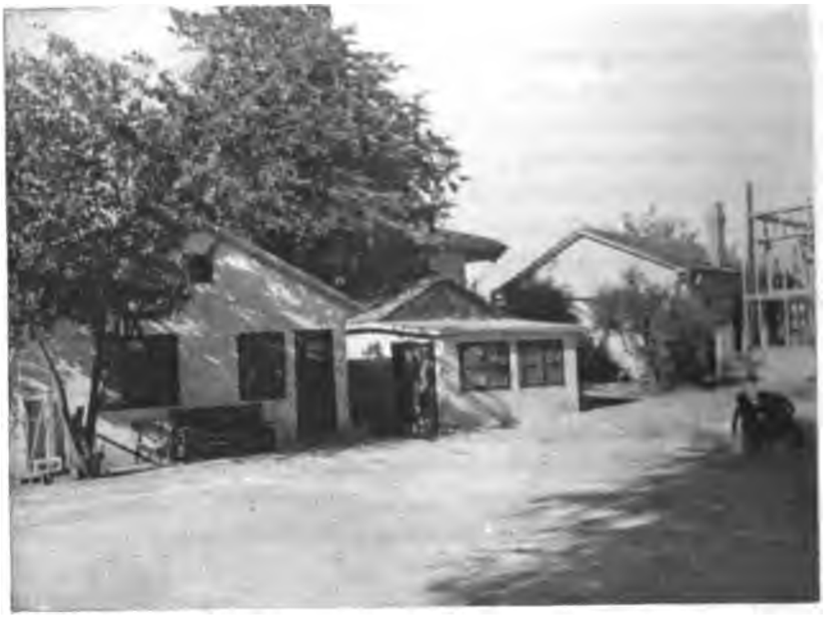

Fig. 1
The Tirana Laboratory (left to right):
a. Malaria Research Laboratory, *b.* Insectary, *c.* Entomological Laboratory, *d.* Corner of Big Cage (under construction).

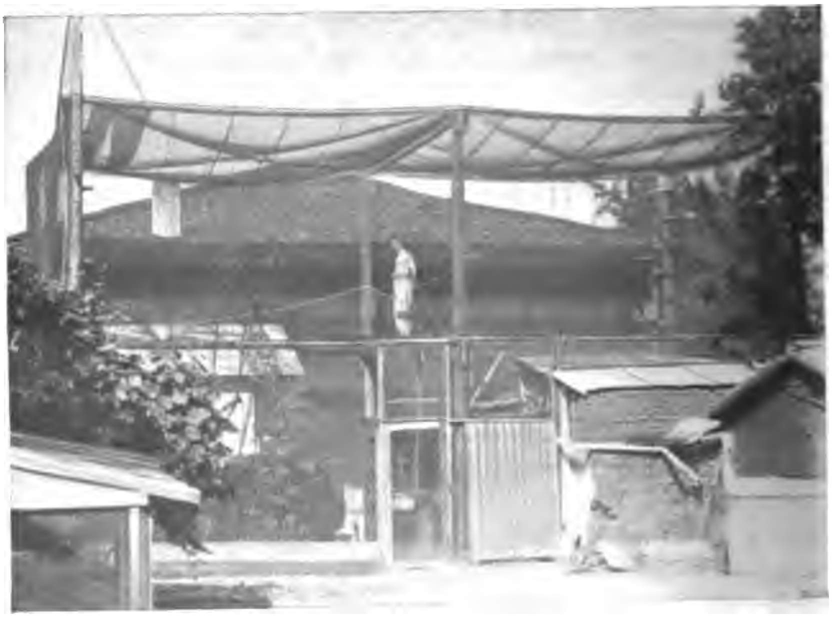

Fig. 2
The Big Cage

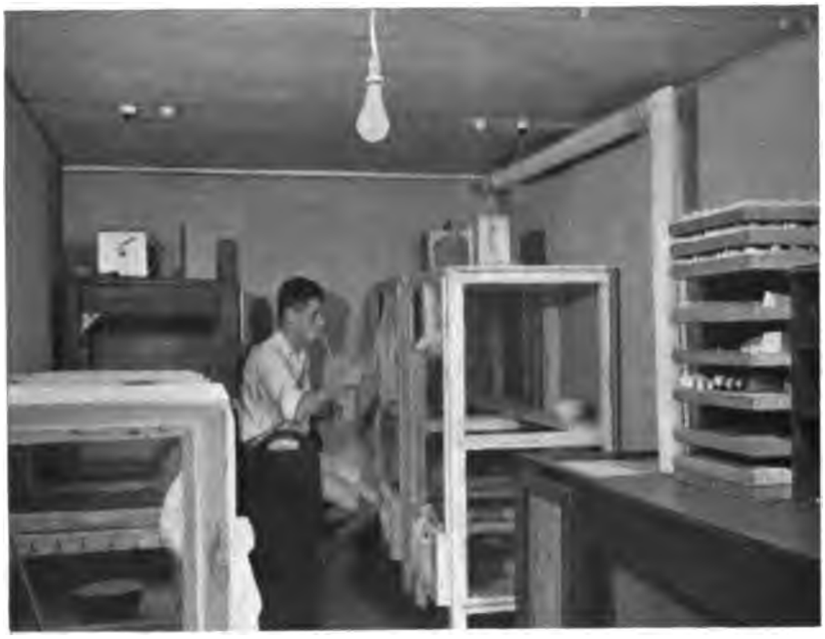

Fig. 3
The subterranean room with controlled lighting, temperature and humidity. Shows type of cage used for anopheline colonies

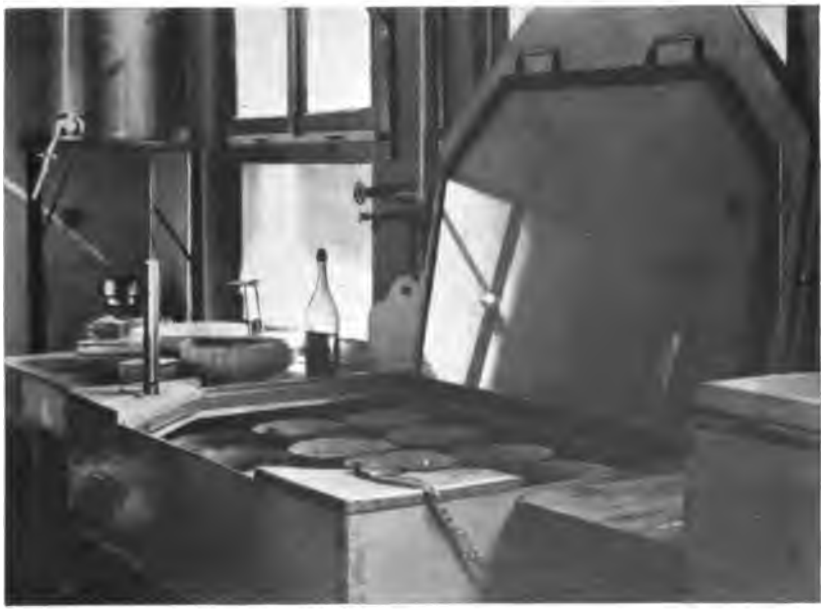

Fig. 4
Water bath (after Boyd) with floating larva pans and thermostatic control

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L. W. HACKETT

Méthodes biologiques de lutte antipaludéenne

Estratto dalla "*Rivista di Malariologia*", - Anno XIV (1935), Sez. I, Suppl. al N. 6

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PROPRIETÀ LETTERARIA

INSTITUT DE MALARIOLOGIE – ROME

Méthodes biologiques de lutte antipaludéenne

Dr. L. W. **Hackett**

2ème CONFÉRENCE

(Cours international de Malariologie, Rome 1935)

Pour la lutte biologique contre des insectes nuisibles, on a reçours généralement à quelque ennemi naturel. Dans le cas des anophèles, par exemple, nous avons découvert un petit poisson, le Gambusia, qui dévore leurs larves. C'est un ami très utile, qui travaille à titre gracieux. Dans certaines régions où les eaux stagnantes constituent les gîtes à larves, le paludisme a pu être supprimé grâce au seul gambusia. En règle générale cependant, le début de son action au printemps est lent et une ou deux générations de moustiques peuvent voir le jour avant que les poissons ne soient en assez grand nombre pour être utiles. Leur action n'est jamais très efficace dans les eaux courantes et ils ne peuvent s'attaquer aux larves lorsque la végétation est dense. Le professeur FERMI a proposé de leur adjoindre, dans les lacs et lagunes, des carpes, ce qui serait très utile car celles-ci dévoreraient les plantes aquatiques permettant ainsi aux gambusias de s'attaquer aux larves. Mais les carpes sont recherchées et détruites par les pêcheurs...

Differences biologiques entre les races d'*Anopheles maculipennis*.

Je me propose de vous parler d'une intervention biologique d'un type tout différent. Nous possédons certaines connaissances sur les influences et les conditions naturelles qui déterminent le nombre des anophèles, leur contact avec l'homme et, par conséquent, le degré de transmission du paludisme. Certains facteurs influencent le comportement des insectes adultes, d'autres leurs stades aquatiques. Nous pourrions nous imaginer que par des mesures purement biologiques ou, comme certains préfèrent, « naturalistiques », les anophèles pourraient être écartés des habitations ou que les conditions de reproduction pourraient être modifiées de façon à ce que des races inoffensives remplacent des races dangereuses.

Nos connaissances sur les activités déployées par les anophèles dans la nature sont faites presque toutes de conjectures et de déductions. Nous voyons essaimer ces insectes à de rares occasions au moment précis où le jour disparait et nous les surprenons souvent, grace à nos lampes de poche, en train de s'alimenter; mais ce qui se passe en plein air au cours de la nuit reste, en grande partie, un mystère pour nous.

Le lever du soleil est le signal d'une fuite éperdue en quête d'un abri et un certain nombre d'anophèles deviennent l'objet de nos observations attentives, alors qu'ils se reposent dans une immobilité complète pendant 12 à 14 heures, c'est à dire jusqu'au moment où l'obscurité revient pour leur rendre leur liberté. Nous avons appris à colorer les moustiques de différentes couleurs, de sorte que nous pouvons les reconnaître si nous les retrouvons. Toutefois, c'est la méthode des précipitines pour déterminer le sang ingéré, qui nous a permis de ne plus dépendre essentiellement des insectes en captivité et des conditions artificielles de laboratoire. En analysant le sang contenu dans l'estomac du moustique, nous pouvons savoir quel animal il a piqué récemment.

Nous avons déjà recueilli des données sur les derniers soupers de milliers d'anophèles d'espèces et de races différentes et ces dernières, de même que les espèces, se différencient les unes des autres per le degré ou l'ordre de préférence qu'elles marquent pour divers hôtes possibles. Dans les vallées bien irriguées et les hautes terres de la Toscane, où les variétés de *Anopheles messeae, melanoon* et *typicus* abondent, il est extrêmement rare de capturer, même dans les chambres à coucher, un specimen dont l'estomac contienne du sang humain. Dans ces régions, ainsi que dans toute l'Europe, à quelques exceptions près, ces races se nourrisssent habituellement sur les animaux domestiques de grande taille. D'autres formes ont des préférences moins marquées. Dans l'Italie méridionale et dans la Sardaigne où le *labranchiae* prédomine, 10 à 20 % de ces anophèles se nourrissent de sang humain tandis qu'en Macédoine grecque un tiers des *elutus* capturés se trouve avoir piqué l'homme. L'étude la plus approfondie qui ait été publiée récemment sur ce sujet, est celle de BARBER et RICE; elle a trait a une région très impaludée de la Macédoine grecque où l'on rencontre réunis l'*elutus*, le *typicus* et le *messeae*. Les auteurs ont obtenu plus de 13.000 épreuves sanguines positives pour ces variétés; le nombre des *elutus* dont l'estomac contenait du sang humain était cinq fois plus élevé que celui des *maculipennis*.

Instinct hémophage et infection.

Si ce fait est exact, nous devrions trouver plus d'*elutus* que de *messeae* ou de *typicus* infectés par les parasites du paludisme. Il faut des soins et de la dextérité pour extraire les glandes salivaires d'un moustique et vérifier si elles contiennent ou non le parasite paludéen. Seul un malariologiste peut apprécier le travail que représente une semblable dissection et l'examen de 37,000 anophèles ; cependant BARBER et RICE nous ont donné le petit tableau ci-dessous, établi après trois ans de patientes recherches. Il est rare que les résultats d'un matériel d'investigations aussi imposant, puissent s'exprimer de manière aussi brève :

elutus . . .	disséqués :	22.200	infectés :	1,29 %
maculipennis	»	14.713	»	0,07 %

Pour chaque *maculipennis* infecté, 19 *elutus* infectés ont été observés Les *messeae* et les *typicus* étaient évidemment les victimes plutôt que les agents du paludisme endémique. Il est naturel que les auteurs aient eu l'idée que les *maculipennis* pouvaient ne pas contracter l'infection paludéenne aussi facilement que l'*elutus* car, à ce sujet, les espèces diffèrent. Le *superpictus*, par exemple, peut être infecté par le paludisme presque trois fois plus facilement que le *maculipennis*. Néanmoins, lorsque les auteurs firent piquer le même porteur de parasites paludéens en même temps par un grand nombre d'*elutus* et de *maculipennis*, les taux d'infection après un seul repas furent presque identiques (20 %).

Par conséquent, le paludisme n'existe pas nécessairement là où l'on rencontre un grand nombre de moustiques susceptibles de s'infecter, mais seulement dans les régions où se trouve l'espèce anophélienne qui pénètre avec insistance dans les habitations pour se nourrir sur les êtres humains. Dans la Macédoine orientale, c'est l'*elutus* qui propage le paludisme, alors que le *messeae* et le *typicus* n'ont pour ainsi dire aucune part dans cette transmission ; en effet, dans une région où nous pouvons dire que le paludisme est général parmi les habitants, pas un insecte sur 1000 de l'une de ces deux races ne s'infecte. De même, dans l'Italie méridionale et en Espagne, c'est le *labranchiae* et non le *melanoon* que l'on trouve avec lui, qui est le vecteur du paludisme, tandis qu'aux Pays-Bas, c'est l'*atroparvus* et non le *typicus* ou le *messeae*.

Avant que MARTINI et ZOTTA n'aient accompli leur enquête en Roumanie, en 1933, on ne connaissait aucune région, dans une partie quelconque de l'Europe, où le *messeae*, le *typicus* ou le *melannon* fut associé au paludisme endémique. On a trouvé maintenant que quelques villages impaludés situés juste en amont du delta du Danube se trouvaient dans une région où l'on ne rencontrait que le *messeae*. Dans une autre région peu étendue c'est le *typicus* qui est accusé de transmettre l'infection. Le paludisme était bénin et du type *vivax*, mais il était endémique ; en d'autres termes, il se perpétuait d'année en année dans les conditions locales normales d'anophélisme et des êtres humains. L'année suivante (1934) Barber et moi-même nous découvrîmes des conditions analogues en Russie et d'autres exemples ont été cités ici et là de races soidisant inoffensives que l'on a trouvées propageant le paludisme. Ceci démontre simplement que les anophèles qui normalement se nourrissent sur le bétail s'attaquent, dans certaines circonstances, fréquemment et en grand nombre, à l'homme.

Deux types de paludisme en Europe.

Au point de vue épidémiologique, il existe évidemment deux types de paludisme en Europe. L'un est dû à la présence d'espèces ou de races d'anophèles qui se nourrissent généralement sur l'homme et sont dangereux en proportion exacte de leur nombre ; l'autre dépend des conditions locales — sociales ou de milieu — qui permettent un certain degré de contact entre l'anophèle et l'homme, contact qui est normalement supprimé grâce aux influences biologiques naturelles, dans les communautés agricoles établies depuis longtemps et en cours de développement. Il n'existe aucun rapport entre le nombre de ces anophèles et la fréquence du paludisme.

Le pronostic sur le cours du paludisme dans ces deux cas varie aussi complètement et nécessite naturellement un double programme de lutte.

Dans le premier cas, les anophèles, en quête de sang, pénètrent instinctivement, et dans toutes les circonstances, dans les habitations, sans se préoccuper des animaux domestiques. C'est partout la caractéristique de toutes les espèces dangereuses.

La fréquence du paludisme est liée directement à l'existence et à l'importance des gîtes à larves, de types spéciaux, produisant ces moustiques ; elle a fort peu de rapport — si même elle en a — avec la prospérité ou l'alimentation des habitants, le genre de logements et le nombre et les conditions dans lesquelles se trouve le bétail.

On conçoit l'existence de mesures biologiques qui pourraient interrompre la transmission du paludisme sans diminuer le nombre des moustiques, mais il reste encore à les découvrir. Notre défense n'est pas biologique, mais elle est organisée par une intervention mécanique directe: l'élimination des gites à larves et la protection des habitations contre les moustiques. Seules deux races de *maculipennis* sont constamment en rapport avec ce type de paludisme : l'*elutus* et le *labranchiae*, tandis que l'*atroparvus* peut être considéré comme un membre du groupe dans une partie de son habitat, en particulier en Espagne et au Portugal.

Dans le deuxième cas, la fréquence du paludisme ne dépend que peu et indirectement du nombre d'anophèles produits dans la localité, mais principalement du degré de déficience ou d'efficacité d'une barrière naturelle protectrice existante entre les anophèles et l'homme. Ainsi dans des régions parfaitement comparables quant à la densité des anophèles, nous pouvons rencontrer toute la gamme du paludisme, depuis les cas sporadiques jusqu'à l'endémie la plus intense.

Les facteurs qui déterminent la deviation effective de certaines races.

Quels sont les facteurs qui interviennent dans la « déviation » animale effective et obligent les anophèles zoophiles à piquer en grand nombre un hôte moins apprécié, tel que l'homme ? Comme dans le cas des puces de rats et de la peste humaine, le nombre insuffisant d'un certain hôte peut conduire un insecte qui se nourrit de sang, vers une autre proie. Ainsi la rareté d'animaux domestiques ou une production excessive d'anophèles peuvent avoir le meme effet qui est de précipiter ces derniers en nombre inusité sur l'homme, ou même, si le contact habituel n'est pas augmenté, il peut etre rendu plus dangereux par la présence d'une quantité anormale de porteurs de gamétocytes qui rompront momentanément l'équilibre. De semblables conditions sont favorisées par des troubles sociaux et des désastres, par une agriculture primitive ou mal equilibrée et par le stage des pionniers de la civilisation parce que ces état de choses ont pour conséquence, pendant une certaine période, un niveau de vie précaire, un production illimitée d'anophèles et une pénurie de bétail.

La guerre mondiale, par exemple, a réveillé le paludisme dans de nombreuses regions oú il était inconnu depuis des générations. En Tchécoslovaquie, non seulement la tierce bénigne (*Plasmodium vivax*), mais également la tierce maligne (*P. falciparum*) ont fait leur apparition, avec un degré élevé de transmission facilitée par la désorganisation de la vie rurale et le retour de soldats infectés. Le paludisme disparut ensuite automatiquement au cours des années suivantes — tout d'abord le *falciparum*, puis le *vivax* — sans qu'aucune mesure préventive spécifique n'ait été prise. Nous ne possédons pas de données sur les types d'anophèles de Tchécoslovaquie, mais nous sommes en droits de supposer, sans risquer de nous tromper beaucoup, qu'il s'agit des types d'eau douce que l'on rencontre habituellement sur le continent : l'*A. messeae* et le *typicus*, qui sont maintenant retournés aux animaux comme hôtes préféreés.

Dans la Russie centrale, nous rencontrons de Moscou à Astrakhan, dans le bassin du Volga, un degré de paludisme endémique peu prononcé, entretenu par le *messeae* et le *typicus*. Tout ce que nous savons sur ces deux espèces d'anophèles, nous démontre que dans les conditions qui règnent dans l'Europe occidentale, elles sont actuellement inoffensives. Elles sont relativement peu dangereuses même dans les Balkans, où elles se rapprochent peut-être des frontières de leur *habitat* climatique. Nous sommes arrivés à la conclusion que ces espèces sont inoffensives en raison de la quantité de bétail stabulé actuellement dans les régions d'agriculture intensive et de développement de l'élevage. D'après nos observations en Russie et celles de MARTINI et ZOTTA en Roumanie, nous avons l'impression nette qu'à ce point de vue la population rurale de ces pays est retardée. Il n'est pas impossible que la Russie, en dépit de ses progrès rapides relativement à l'introduction du bétail et l'adoption de méthodes agricoles modernes, se trouve encore dans des conditions très analogues à celles qui caractérisaient le reste de l'Europe à l'époque où florissait le paludisme. Le paludisme endémique, bien qu'il soit très répandu en Russie, ne peut pas être qualifié d'intense, et consiste uniquement en tierce bénigne. Nous pouvons donc prévoir un développement progressif de la vie rurale créant une situation qui détournera des habitations les anophèles sans les supprimer, mais dans laquelle le paludisme disparaîtra.

Au point de vue du paludisme, la plus importante modification des formes primitives de l'agriculture, est l'introduction des cultures alternées. Tous les trois ans les champs sont plantés de légumes pour fertiliser le sol, ce qui aurait pour résultat un excès de fourrage si l'équilibre économique n'était pas rétabli par une augmentation du bétail. Ainsi l'élévage se développe en raison d'une nécessité économique et le fourrage donné dans les étables remplace le pâturage naturel. Ceci crée les meilleures conditions pour la « déviation » des anophéles zoophiles et, en Pologne, par exemple, où les conditions étaient très semblables à celles de la Russie, nous voyons que le paludisme a pour ainsi dire disparu actuellement grâce à une agriculture bien equilibrée et à l'absence de tout anophèle autre que le *messeae* et le *typicus*.

Il existe une condition sociale qui est habituellement liée à une propagation intense du paludisme et c'est la phase de début de l'occupation et du développement d'une région. Un des meilleurs exemples de ce genre en Europe nous est offert par le delta de l'Ebre, en Espagne, qui, il y a peu de temps encore, n'était qu'un désert et qui a été récupéré depuis pour la culture du riz sur une large échelle. La transformation des terres, l'immigration d'ouvriers infectés par de souches ésotiques de parasites, l'absence de logements jointe à une surpopulation et, enfin, la pénurie de bétail, créérent une situation très sérieuse au point de vue du paludisme. A l'heure actuelle, une agriculture scientifique, avec cultures alternées, la production de fourrage et l'augmentation du bétail a déjà reduit de beaucoup le contact des anophèles avec l'homme et, par suite, le paludisme. L'*atroparvus*, que se nourrit sur le bétail, est remplacé peu à peu par le *melanoon*, — type caractéristique des rizières, — qui pénètre encore moins que l'*atroparvus* dans les habitations. Une grande importance a été attribuée au fait que la région de l'Ebre est deltaique, mais je doute qu'il existe un problème du paludisme des deltas en tant que phènomène particulier. Chaque delta constitue un problème paludéen indépendant avec ses conditions spécieles, et l'heureuse solution de la plupart de ces problèmes en Europe, est due à la race du *maculipennis* qui a fini par supplanter les autres.

La zooprophylaxie.

Dans les exemples comme celui ci-dessus, la question du type d'anophèle est capitale. On ne peut ignorer le développement de très nombreux *labranchiae* et d'*elutus* dans les « bonifications» de l'Italie méridionale tel qu'on l'a fait pour les anophèles de l'Italie septentrionale où l'on avait affaire à des races zoophiles. Les barrières constituées par les animaux ne sont jamais inutiles, mais lorsqu'il s'agit d'anophèles dangereux qui sont vecteurs par instinct et non en raison des conditions locales, les animaux ne *détournent* pas autant les anophèles, qu'ils n'apportent un grand nombre d'hôtes réfractaires, de sorte que de nombreuses piqûres virtuellement infectieuses ne le deviennent plus. Ainsi, sur 54 *elutus* infectés, dont Barber a pu déterminer la provenance du dernier repas sanguin, 63 % avaient piqué des animaux domestiques. Certains auteurs ont une confiance presque illimitée dans les barrières animales, tel Falleroni, qui estime que l'élevage du bétail, intelligemment subventionné, secondé par la construction scientifique de porcheries et d'étables disposées de facon appropriée, supprimeraient complètement le paludisme. De même qu'une quantité de rats diminue les probabilités de peste humaine, de même une grande quantité de vaches, chevaux et porcs signifierait, selon l'école « zooprophylactique », la cessation de la transmission du paludisme.

Si je ne me trompe, c'est Schüffner qui le premier a démontré l'inexactitude de ce raisonnement. Il avait observé que les buffles (d'eau) stabulés à Java attiraient l'*A. sinensis* 50 beaucoup plus que l'homme et lorsqu'en 1923 il se trouva avec WALCH en face d'un paludisme provoqué par le *sinensis*, sur la côte est de Sumatra, où les buffles n'étaient pas enfermés la nuit, il fit construire un certain nombre d'étables protectrices. A sa grande surprise, cette mesure n'eut pas la moindre influence sur les conditions du paludisme. Les anophèles continuèrent de piquer les hommes dans les baraquements, dans la même proportion que les buffles. Reprenant le problème dix ans plus tard, WALCH, utilisant alors la méthode des précipitines, démontra que, contrairement au *sinensis* de Java, la forme que l'on rencontre à Sumatra n'est que légèrement détournée par les animaux. Lorsqu'il n'y avait qu'un petit nombre d'animaux aux alentours, 90 % des *sinensis* capturés dans les habitations contenaient du sang humain, mais si les animaux étaient nombreux, ce taux était réduit à 83 %. Avec le *maculatus* cependant, qui se nourrit de préférence sur le bétail, la présence d'animaux portait la proportion de 97 à 11 %.

Par conséquent, les barrières animales ne peuvent être utiles dans la pratique que lorsqu'on a affaire à des anophèles qui, par instinct, se nourrissent sur le bétail. Pour autant que nous sachions, l'*elutus* et le *labranchiae* ne sont jamais détournés de manière efficace par le bétail et provoquent toujours le paludisme là où ils existent. D'autre part, il y a de vastes régions où le *messeae*, le *melanoon*, le *typicus* et *l'atroparvus* sont détournés des habitations en nombre suffisant pour interrompre la transmission du paludisme. La déviation naturelle n'a pas besoin d'etre completée pour être efficace. Des nombreux anophèles qui piquent l'homme, un faible pourcentage seulement finira par transmettre le parasite du paludisme à un autre individu. La nature arrive à ses fins par la prodigalité de ses moyens. Pour que le paludisme puisse se propager et se maintenir, il est nécessaire qu'un nombre considérable d'anophèles pénètrent continuellement et régulièrement dans les habitations, chose impossible pour les 3 ou 4 races susdites, étant donné le degré d'attraction que les étables exercent sur elles.

De ces races, l'*atroparvus*, ainsi que nous l'avons remarqué, est parfois un vecteur du paludisme, pénétrant dans les maisons par instinct, alors que d'autres fois, il est inoffensif, étant facilement détourné par l'attrait des étables.

Dans le domaine général de l'anophélisme sans paludisme, nous avons (ainsi que le fait remarquer MARTINI) un problème d'« *atroparvisme* sans paludisme ». Le comportement de l'*atroparvus* résulte toujours de forces instinctives et des conditions de milieu, comme c'est le cas pour tout autre insecte, ce qui sera expliqué un jour. Nous devons cependant examiner maintenant un des aspects de ce comportement, car il a donné naissance à une théorie de lutte antipaludéenne par des mesures biologiques qui ne correspond guère aux faits, tels que nous les connaissons actuellement.

Paludisme et habitation.

Jusqu'au moment où l'on a découvert les races du *maculipennis*, l'opinion générale voulait que les habitations sombres, humides et mal aérées exerçassent un attrait spécial sur les anophèles, qu'on y trouvait souvent se reposant en plus grand nombre que dans les autres, et devinssent de ce fait des centres de transmission du paludisme.

En 1916, par examplе, le Col. James remarquait la présence de *maculipennis* dans certaines maisons sur la côte orientale d'Angleterre. Ces insectes sont restés pendant des semaines ou des mois dans les chambres sans sortir pour pondre, et provoquèrent plusieures cas de paludisme. James supposait que cela dépendait du caractère des maisons qui étaient agréables aux moustiques, de sorte qu'elles devenaient les soi-disantes « maisons à paludisme »; mais nous savons aujourd'hui que celà est dû a la présence en Angleterre près de la mer d'une race *atroparvus* laquelle n'hiberne pas. Elle pénètre dans toutes les maisons, mais seulement en certains cas elle rencontre des porteurs de gamétocites et s'infecte. Dès que ses ovaires ne fonctionnent pas, elle peut rester dans la maison un temps suffisant pour infecter presque toute la famille, en donnant ainsi un caractère focale au paludisme. Mais personne n'avait jamais remarqué que ce phenomène ne se verifiait pas dans l'Angleterre centrale, où il y a une autre race qui entre en hibernation complète dans des abris inhabités. Les differences ne sont pas à chercher dans les maisons — leur type de construction, l'humidité, l'illumination — mais seulement chez les anophèles.

C'est vrai que les moustiques ont des abris préférés, mais on sait que les endroits où les anophèles se réunissent en masse pendant le jour pour y chercher un abri et faire leur digestion, ne fournissent aucune indication sur leurs déplacements nocturnes, alors qu'ils propagent le paludisme. Les *messeae*, *melanoon* et *typicus* aiment également les endroits frais et humides et pénètrent dans les habitations pendant le jour, mais ils piquent la plupart du temps dans les étables. Les *superpictus*, au contraire, piquent dans les maisons et préfèrent se reposer dans les étables. Barber a démontré que la distribution des infections infantiles dans les villages de Macédoine, n'a aucun rapport avec l'agglomérations des insectes pendant le jour et de Buen, en Espagne, a prouvé que les maisons dans lesquelles se produisaient des cas de paludisme ne sont pas celles où l'on capture le plus grand nombre de moustiques. En ce qui concerne la transmission du paludisme, nous croyons, par conséquent, que la seule différence importante entre une maison et l'autre est la présence ou l'absence de protection mécanique. La construction de chambres à coucher sèches, claires et bien aérées, constitue une excellente mesure d'hygiène, mais nous ne sommes pas encore disposés à la considérer comme une des mesures biologiques préventives contre la transmission du paludisme.

L'*habitat* larval.

Nous avons dit que l'*elutus* et le *labranchiae* piquent l'homme aussi volontiers que les animaux et, par conséquent, nous ne pouvons nous attendre à obtenir des résultats très satisfaisants avec la zooprophylaxie dans les régions où ces anophèles prédominent. Etant donné que l'attraction exercée par les étables n'est pas suffisante pour interrompre la transmission du paludisme, nous devons envisager une attaque de l'insecte à son stade larvaire. Nous savons que chaque race s'est adaptée à des types de gîtes à larves spéciaux. Les trois vecteurs du paludisme pondent de préférence dans des eaux légèrement salées et les non-vecteurs dans des eaux douces, mais il existe un certain chevauchement. Nous observons des signes de rivalité entre les formes, l'une chassant l'autre lorsque les conditions changent sous l'influence des saisons. Etant donné que dans la nature tout se multiplie au maximum permis par les conditions locales, l'insecte le mieux adapté ne pourra étendre son domaine qu'au détriment d'autres formes. Dans l'intérieur de la Vénétie et dans le delta du Pô, il y avait autrefois une quantité d'*elutus*, mais l'irrigation et les modifications complexes apportées par l'agriculture au cours des vingt dernières années, ont eu pour effet de remplacer, dans une large mesure, l'*elutus* par l'*atroparvus* lequel, dans cette région, ne transmet pas le paludisme. D'autre part, on a observé que l'*atroparvus* était remplacé à son tour par le *messeae* et le *melanoon*, qui sont des especes typiques d'eau douce.

Les Pays-Bas, où l'on récupère constamment des terres sur la mer, nous offrent un des exemples les plus frappants de substitution d'une race par une autre. Pendant les dix premieres années, ou même davantage, après l'exclusion de la mer, les gites à larves contiennent de l'eau légèrement salée et produisent un grand nombre d'*atroparvus*, vecteurs du paludisme. Du fait que l'irrigation adoucit l'eau et que par les nouveaux polders, les anciens se trouvent de plus en plus éloignés de la mer, l'*atro-*

parvus disparait et fait place au *messeae*, ce qui aboutit à une disparition graduelle, mais totale du paludisme.

Cette substitution de races peut-elle s'accomplir artificiellement ? Nous faisons actuellement des expériences à ce sujet en Albanie. Tirana, la capitale, est de facon naturelle très impaludée, grâce aux nombreux *superpictus* produit par la rivière de même nom, cours d'eau torrentieux, au lit graveleux qui offre à cet anophèle les meilleures conditions de reproduction. Nous avons maintenant entrepris de détourner complètement en été le cours de la rivière Tirana, la faisant passer par les canaux d'irrigation qui ont dû etre de beaucoup agrandis et multipliés pour contenir cette quantité d'eau supplémentaire. Nous espérons ainsi assécher le lit de la rivière et supprimer la production de *superpictus*. Cependant, si le système d'irrigation crée, entre les mains de paysans ignorants, des gîtes à larves de *messeae* et de *melanoon*, nous ne croyons pas nous en effrayer outre mesure, car nous savons que ces races contribuent peu à la propagation du paludisme dans cette région. Nous avons observé qu'en Italie le paludisme est généralement une maladie du littoral, mais qu'à deux régions littorannéennes, à Viareggio et aux alentours de Naples, il n'y a pas de paludisme et une brève étude nous a montré, que cet état de choses provenait des gîtes à larves à eau douce qui produisent le *typicus* et le *melanoon* au lieu du vecteur de l'infection, le *labranchiae*, qui préfère l'eau saumâtre. A Naples, par suite des sources abondantes, l'eau est naturellement douce ; à Viareggio les marais sont séparés de la mer par des ecluses automatiques de Zendrini qui empechent l'eau de mer de pénétrer dans ces marais, alors que l'eau de ces derniers peut s'écouler dans la mer. C'est certainement ce fait qui a produit la modification du type de population anophélienne, remplacant le dangereux *labranchiae*, que l'on trouve encore au nord et au sud de Viareggio, par le *melanoon* inoffensif. Ceci a permit l'etablissement d'une Station balneaire de premier ordre avec grande affluence de monde pendant l'été.

Une situation très analogue a été observée à Durazzo, le port de l'Albanie, où une grande lagune à eau saumâtre produisait de nombreux *elutus*. Ici, étant donné l'absence de rivières d'eau douce venant se jeter das la lagune, il n'a pas été possible de transformer celle-ci en un lac d'eau douce au moyen des écluses Zendrini. Nous avons décidé, par conséquent, d'augmenter la teneur en sel du lac, jusqu'à ce que l'*elutus* ne puisse plus s'y reproduire, c'est à dire jusqu'à ce que l'eau contienne 2 % de sel. Les écluses automatiques furent disposées de telle façon que l'eau de mer pouvait pénétrer dans la lagune à marée haute, mais ne pouvait en sortir. Dans l'interval de deux ans la teneur en sel de cette vaste lagune d'une superficie d'environ 27 km^2 avait augmenté suffisamment par cause de l'evaporation pour empêcher complètement le développement des anophèles.

Ceci pourrait presque être considéré comme une mesure antilarvaire plutôt que biologique, mais son efficacité est probablement due surtout aux modifications apportées à la végétation. Les larves d'*elutus* disparurent complètement avant que la teneur en sel n'ait atteint la dose mortelle minimum de 20 pour 1000. Le plancton et les algues étaient sans doute plus sensibles au sel que les larves. La destruction et la décomposition de la végétation rendirent alors l'eau impropre au développement des anophèles.

Le type de végétation et, par suite, dans une large mesure, l'espèce anophélienne des gîtes à larves ordinaires, sont fonction de la profondeur de l'eau, et la température dépend probablement du même facteur. Dans l'eau peu profonde, la végétation est apte à être submergée et horizontale, tandis que dans l'eau plus profonde est verticale et émergeante; lorque l'eau atteint plus de deux mètres de profondeur, il n'y a habituellement plus de végétation du tout et point de larves. Ainsi le dragage des bords du lac de Paola dans les Marais Pontins, a créé une eau profonde sur les bords revétus et a diminué la végétation et le développement des anophèles. Les fluctuations du niveau de l'eau changent fréquemment le type de végétation et suppriment souvent les gîtes à larves. A Fiumicino, par exemple, l'utilisation de l'eau du lac de Porto pour l'irrigation et son remplaçement par de l'eau du Tibre amenée au moyen de pompes, produit un changement continuel du niveau du lac, ce qui a détruit complètement, en moins de trois ans, toute la végétation flottante. Il n'y existe plus de gîtes à larves et il n'est pas nécessaire de pétroler ni d'utiliser le vert de Paris.

En règle générale, la bonification, c'est-a-dire l'assèchement des marais par le drainage, l'adjonction de chaux et d'engrais dans le sol, l'introduction de l'irrigation, produisent des modifications subtiles dans l'eau de surface, qui affecteront graduellement les races de *maculipennis* dont les gîtes se trouvent dans la région. Dans les Marais Pontins, nous avons observé, au cours des deux dernières années, un changement progressif du type d'anophèles capturés dans les abris. L'*elutus* et le *labranchiae*, les principaux ennemis des fermiers de l'Europe méridionale, ont diminué de nombre lentement par rapport au *messeae* et au *melanoon* et nous sommes peut-être les témoins et l'action progressive d'une modification biologique qui, en fin de compte, pourra être tout aussi efficace pour la disparition du paludisme que l'élimination de celui-ci par le drainage et le revêtement de canaux de surface qui servaient de gites à larves.

Vous ne devez pas croire que seul l'*A. maculipennis* comprenne des races ayant des habitudes d'alimentation et de reproduction différentes. Il n'existe que 12 ou 14 anophèles dangereux dans le monde et l'on a déjà rapporté que plusieurs d'entre eux semblent se comporter différemment suivant les pays. Les *ludlowi*, *hyrcanus*, *maculatus* et *pseudopunctipennis*, pour n'en citer que quelques-uns, sont vecteurs dans un endroit et pas dans un autre. En Afrique, le *gambiae* et le *funestus* se reproduisent dans des conditions tout à fait différentes suivant les régions. Voici un champ d'étude très important. Néanmoins, on ne peut s'attendre à ce que la nature ait été assez aimable pour revêtir les oeufs des diverses races de couleurs différentes, comme elle l'a fait pour les races d'*A. maculipennis*, afin de nous permettre de les distinguer.

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Dr. L. W. Hackett

1ère CONFÉRENCE

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Les Paradoxes du Paludisme.

Lorsque **Grassi** et ses collaborateurs eurent prouvé, en 1898, que le paludisme humain était transmis par une certaine espèce de moustiques appelés *Anopheles*, **Grassi** estima que ces derniers pouvaient être considérés dorénavant comme *le spie della malaria* (les espions du paludisme). En d'autres termes, partout où l'on trouvait des anophèles, on aurait pu rencontrer également du paludisme. Cependant, on découvrit plus tard qu'il existait six espèces européennes d'anophèles et que seules trois d'entre elles — les *maculipennis*, les *elutus* et les *superpictus* — pouvaient transmettre le paludisme. Peu de temps après, **Celli** et d'autres observèrent que même le *maculipennis* n'était pas un vecteur partout où on le rencontrait. En effet, **Meigen** qui dénomma *maculipennis* l'espèce, n'était absolument pas convaincu qu'il piquât l'homme, car dans la région du Luxembourg où il l'étudia — ainsi que le *bifurcatus* — cet anophèle avait peu de contact avec les êtres humains. Une étude plus approfondie révéla que là où le *maculipennis* transmettait vraiment le paludisme, son comportement avait quelque chose d'irrationnel. Le nombre de *maculipennis* n'était pas toujours en proportion directe avec l'intensité de l'endémie. En fait ainsi que **Celli** l'a écrit il y a plus de 25 ans, ce nombre est souvent en proportion inverse.

Ceci n'était qu'un seul des nombreux paradoxes touchant le paludisme, connus des premiers malariologistes. La relation entre le *paludisme* et les *marais* (paludi) n'existait pas toujours. En effet, en Italie la plupart du paludisme ne se rencontrait pas dans les marais de la vallée du Pô, mais bien dans les régions accidentées telles que la Sardaigne, la Basilicate et la Sicile. Une autre anomalie consistait dans le fait qu'en Europe septentrionale le paludisme précédait chaque année la saison anophélienne au lieu de la suivre. En Angleterre, dans les Pays-Bas, en Allemagne et en Russie, le point culminant du paludisme se plaçait en mai et celui de l'anophélisme en juillet.

La croyance des premiers malariologistes en la théorie qui voulait que le paludisme ne puisse être transmis que par les moustiques, a parfois été ébranlée par ces anomalies, mais depuis lors tout a été expliqué de manière satisfaisante. Le paludisme des régions accidentées, soi-disant paradoxal, était dû aux moustiques dont les gîtes à larves se trouvent dans les eaux courantes ; en réalité, étant donné la distribution de cette maladie dans le monde entier, le mot « paludisme » est aussi inapproprié que le mot « malaria » lui-même. L'apparition du paludisme au début du printemps fut reconnue comme attribuable aux moustiques de l'année précédente. Une des souches principales de *Pl. vivax* de l'Europe septentrionale reste à l'état latent pendant 8 à 10 mois et provoque ensuite un accès primaire au début du printemps.

Mais le paradoxe de l'anophélisme sans paudisme resta une énigme pendant plus d'un quart de siècle, énigme qu'il était indispensable de résoudre étant donné qu'elle paralysait le travail sur la prévention du paludisme.

Il semblait illogique à un grand nombre de personnes de dépenser des sommes importantes du trésor public pour effectuer des essais imparfaits en vue de diminuer les gîtes des moustiques, si le nombre des anophèles avait si peu de rapport avec le paludisme. La lutte contre le moustique était généralement considérée comme une méthode américaine due au succès remporté par les Américains à Panama, et la Commission du paludisme de la Société des Nations envoya un petit comité aux Etats-Unis pour y étudier les causes du succés des méthodes utilisées dans ce pays. A son retour, le Comité déclara qu'il semblait y avoir très peu de paludisme endémique aux Etats-Unis et que si cette maladie était en régression, cela dépendait plutôt des progrès sociaux et économiques, que des mesures préventives appliquées par les organisations d'hygiène. Je ne crois pas que la Commission ait prîs en considération les quantités considérables de canaux de drainage souterrain construits dans ce pays, ni la protection mécanique des habitations contre les moustiques.

Je me suis occupé de travaux antipaludéens, aussi bien en Amérique qu'en Europe et je suis persuadé que la raison principale du succès remporté en Amérique réside dans le fait qu'on avait affaire dans ce pays à des anophèles ne se reproduisant que dans de l'eau stagnante. Par consequent les fleuves perpétueux à faible courant, en Sardaigne et en Sicile qui sont la source principale de l'anophélisme et du paludisme dans ces régions, ne seraient pas du tout nuisibles et ne seraient pas pris en considération, pour l'application de mesures antilarvaires, dans aucun endroit des Etats-Unis, où le vecteur du paludisme c'est le *quadrimaculatus*. Si les eaux sont mises en mouvement au moyen de canaux de drainage, les anophèles ne les utilisent plus comme gîtes à larves..

En Europe, l'eau à faible courant des grands canaux de « bonification » constitue souvent des gîtes à larves plus prolifiques pour les anophèles que les marais qu'ils remplacent !

Néanmoins, l'existence en Europe de vastes régions à population très dense, où l'on rencontre un nombre prodigieux d'anophèles sans le moindre paludisme, a toujours présenté un obstacle théorique à la lutte antipaludéenne par la destruction de ces insectes. Chacun sentait qu'il pouvait exister une méthode biologique capable d'enrayer la transmission du paludisme, sans qu'il soit nécessaire de réduire le nombre des anophèles. On ne tarda pas à découvrir que pratiquement chaque partie de l'Europe possédait sa population anophèlienne, mais que le paludisme n'était transmis que dans fort peu de régions. Dans le cas du *maculipennis*, son rapport avec le paludisme constituait plutôt une exception que la règle. Il était important, par conséquent, d'étudier les causes de l'anophélisme sans paludisme, soit pour pouvoir imiter le mécanisme naturel encore secret de celui-ci, soit pour examiner au moins la possibilité de produire artificiellement les mêmes résultats.

Théories sur la disparition naturelle du paludisme.

Quelles pouvaient bien être les raisons de la disparition naturelle du paludisme dans d'aussi nombreuses régions d'Europe complétement dissemblables ? Il va de soi que plusieurs théories furent émises. Le prof. FERMI en a publié récemment une longue liste couvrant plusieurs pages. Les malariologistes furent bientôt plus intéressés par les anophèles qui ne transmettaient pas le paludisme que par ceux qui étaient vecteurs. Nous ne pouvons citer ici que quelques exemples génériques de ces théories.

Il y a peu de temps encore, la croyance était largement répandue que dans certaines régions le *maculipennis* était réfractaire à l'infection paludéenne. Dans les rizières de Vercelli et de Toscane, ainsi que dans les régions irriguées du delta du Pô, il était souvent possible de compter jusqu'à 50.000 anophèles dans une seule étable et l'on affirmait que bien qu'ils piquassent fréquemment les êtres humains, ils étaient incapables de transmettre le paludisme. À ce propos, il fut heureusement possible de faire une expérience décisive : lorsque la malariathérapie fut utilisée sur une large échelle contre la paralysie générale progressive, une station fut établie à Rome pour la transmission du paludisme par les moustiques infectés. Afin d'éviter l'utilisation d'insectes déjà infectés naturellement, les anophèles furent recueillis dans des régions à anophélisme sans paludisme et l'on observa qu'ils pouvaient être infectés sans la moindre difficulté.

On suggéra alors que l'homme était peut-être plus résistant au paludisme dans ces régions. On fit observer que le paludisme avait commencé dà disparaître de l'Italie du Nord lorsque les conditions de vie de la population s'améliorèrent.

D'après un vieux dicton « la malaria vien della pentola » (le paludisme vient de la marmite) et l'on croyait que l'un des effets de la prospérité était la suppression du paludisme. On a fait observer que la famine précédait généralement les épidémies du nord de l'Inde. L'essai le plus enthousiaste et, en même temps, le plus désastreux, basé sur cette théorie, a été celui tenté en 1897 par F. Cirio, fabbricant de conserves de fruits. Il établit une colonie agricole de Vénitiens sur les champs vastes et fertiles — mais inhabités — des Marais Pontins. Il avait l'habitude de dire : « *Ma che malaria* ! » (Le paludisme ? Quelle histoire !). « Le paludisme entre par la cuisine. Je vais leur donner de la viande, du vin, du café et des quantités de quinine ». Le Dr. Cardinali, médecin de Terracina, écrivait plus tard : « Il y avait de quoi émouvoir aux larmes de voir ces robustes colons descendre de leurs montagnes rayonnant de santé et être frappés, comme par la foudre, par le paludisme pernicieux, contre lequel ni la nourriture abondante fournie par Cirio, ni la quinine que je distribuais largement, ne parvenait à élever une barrière ». Cet essai n'est peut-être, pas concluant ; mais, en étudiant le terrain, on observe qu'en Europe tout au moins ce ne sont pas les populations vivant dans des conditions économiques défavorables qui souffrent le plus du paludisme endémique.

L'attention fut portée alors sur les facteurs de milieu. En 1903 déjà, Bonservizi avait observé l'attraction des étables pour le *maculipennis*. Il semblait possible que là où se trouvaient de nombreux animaux domestiques, dans des étables « odoriférantes », les *maculipennis* fussent presque complètement détournés des habitations. Il était évident que les moustiques fréquentaient de préférence des endroits sombres, humides, sans courants d'air et qui, en même temps, leur offrent la possibilité de s'alimenter la nuit. Les habitations n'attireraient par conséquent pas les moustiques si elles étaient sèches, aérées et bien éclairées. Un des résultats de la prospérité avait été de mettre à la disposition des populations rurales des habitations plus confortables, et de nombreuses personnes crûrent que cette mesure non spécifique vaincrait le paludisme. D'après l'opinion dominante, le paludisme était une maladie sociale qui persistait grâce à l'ignorance et à la pauvreté et les progrès de a civilisation suffiraient avec le temps à le supprimer de n'importe quelle région. Le professeur **Marchoux** me dit un jour : « Vous, Américains, vous êtes impatients ; vous devez faire les choses en hâte. Nous qui disposons de plus de temps et de moins d'argent, nous croyons que le progrès social et économique des populations supprimera le paludisme avec le temps ».

De fait, les mesures de « bonification » du nord de l'Italie, qui ont été appliquées depuis des siècles, ont supprimé le paludisme sans diminuer le nombre des anophèles. Cette théorie, aussi attrayante soit-elle, n'est plus soutenable. Une investigation, même peu approfondie, montrera que les régions à endémie paludique opiniâtre, sont loin d'être les plus retardées, les plus misérables et les plus ignorantes d'Europe. Il suffit de noter que le paludisme continue à être transmis, année après année, dans la Hollande du Nord, dans certaines parties de l'Allemagne septentrionale, et dans l'intérieur de la Vénétie, alors qu'il a completement disparu du Sud de la France, de Naples et du delta du Nil.

En 1920, **Roubaud** proposa une nouvelle théorie, basée sur la rivalité des anophèles en quête de nourriture. Ceux munis d'organes aigus plus forts pourraient s'attaquer plus efficacement aux animaux domestiques à peau épaisse et tendraient à supplanter les insectes plus faibles, qui ne peuvent piquer que l'homme. Cette puissance maxillaire serait transmise aux descendants de l'heureux groupe et de cette façon naîtrait une race qui ne s'attaquerait qu'aux animaux et ne transmettrait plus de paludisme. Ce fait a son importance, car pour la première fois l'on employait le mot « race » en rapport avec l'*A. maculipennis*. Cette théorie n'a pas été unanimément acceptée, ainsi que le proposait **Roubaud**. Le paludisme n'a disparu de l'Europe septentrionale qu'au cours du siècle passé. et il n'est pas probable que depuis lors une nouvelle race, incapable de se croiser avec la souche dont elle est issue, ait eu le temps de surgir. Nous croyons que des espèces différentes ont des indices maxillaires différents, mais que la même espèce de moustique, qu'elle soit trouvée dans une étable ou dans une chambre à coucher, aura, en moyenne, le même nombre de dents. **Barber**, utilisant la méthode des précipitines, a observé de nombreux moustiques infectés dont l'estomac était plein de sang de vache, ce qui prouve que le même insecte peut piquer — et pique — les animaux et l'homme.

Quelques années plus tard, des observateurs hollandais (**De Buck**, **Swellengrebel** et d'autres) ont décrit deux espèces de *maculipennis* dans les Pays-Bas : un type de petite, et un de grande taille. On les a découvert en mésurant leurs ailes, qui se sont trouvées être proportionnées à la taille du moustique. Les macroptères (ailes longues) avaient comme gîtes à larves des eaux douces et hibernaient complètement en hiver; les microptères (ailes courtes) pondaient dans des eaux saumâtres et n'hibernaient pas. Un changement physiologique s'opérait en automne dans les microptères, de sorte que tout en continuant à rechercher le sang, ce dernier ne stimulait plus les fonctions ovariennes et les insectes n'éprouvaient plus le besoin de pondre. Ils choisissaient pour passer tout l'hiver, une étable ou une habitation chaude. Si l'une ou l'autre d'entre elles se trouvait être infectée, ou si un membre de la famille était porteur de gamétocytes, il en résultait à la fin de l'hiver une infection de presque tous les êtres humains habitant la maison et, par suite, des autres anophèles qui se seraient réfugiés sous le même toit.

Nous possédons ainsi un exemple de différenciation raciale des moustiques, d'où il résulte qu'une seule des espèces est vectrice du paludisme. Ce fait constitue évidemment un cas spécial, étant donné que nous trouvons également des microptères en France, en Belgique et en Italie, où ils ne sont jamais vecteurs du paludisme. En ce qui concerne les différences d'hibernation dans le bassin méditerranéen, il était naturellement tout à fait évident qu'elles n'avaient aucun rapport avec le problème du paludisme.

Les races d'*A. maculipennis*.

En 1926 **Missiroli** et moi-même, nous nous attaquâmes au problème d'une autre manière. D'après nous, la seule raison pour laquelle un anophèle susceptible de transmettre le paludisme n'était pas vecteur, résidait dans son contact insuffisant avec l'homme. Nous entreprîmes donc d'évaluer le degré de ce contact par des méthodes quantitatives, dans des régions hyper-endémiques, épidémiques et non paludéennes. Nous eûmes recours à la méthode des précipitines, établie par **Uhlenhuth** et communément employée actuellement dans les recherches médico-légales pour déterminer la source des taches de sang suspectes. Le résultat fut tel que nous le prévoyions : une proportion élevée des moustiques provenant de régions très impaludées contenait du sang humain, tandis que l'estomac des insectes capturés dans des régions d'anophélisme sans paludisme n'en contenait pour ainsi dire point. Par exemple : à Fiumicino, dans le delta du Tibre, 84% des moustiques capturés dans les chambres à coucher donnèrent un résultat positif en ce qui concerne le sang humain, mais à Valdichiana, en Toscane, pas une *maculipennis* n'a été trouvé jusqu'à présent avec du sang humain dans l'estomac. Cette constatation n'avait aucun rapport avec l'attrait des habitations pour les anophèles, car à Fiumicino les maisons étaient neuves et protégées mécaniquement — bien qu'imparfaitement —, alors qu'en Toscane elles étaient vieilles et sombres. Nous étions persuadés qu'il s'agissait d'une question de race, mais une comparaison des moustiques des deux régions, très éloignées l'une de l'autre, ne révéla aucune différence entre eux. Les recherches se concentrèrent maintenant sur la définition de ces deux races biologiques afin de nous permettre de distinguer entre les « amis » et les « ennemis ». Nous avons invité le professeur MARTINI de l'Institut de Médecine Tropicale de Hambourg, à collaborer avec nous, mais nos études sur les soies des larves et l'hypopygium des mâles eurent peu de succès au début.

En 1924, alors que VAN THIEL mesurait les ailes des moustiques hollandais, FALLERONI, un inspecteur retraité de la Santé Publique d'Italie, faisait une importante découverte, d'après laquelle les femelles de *maculipennis* pondaient un certain nombre d'oeufs d'espèces différentes. Il décrivit deux types généraux : des oeufs foncés et des oeufs clairs et les nomma *messeae* et *labranchiae* (d'après deux de ses amis du Service de la Santé, les Docteurs MESSEA et LABRANCA). Cependant, ce ne fut que 7 ans plus tard que MARTINI, MISSIROLI et moi-même songeâmes à appliquer cette observation de façon pratique. Nous commencâmes par recueillir des oeufs de moustiques de toutes les parties de l'Europe et nous en trouvâmes 6 différentes sortes, qui avaient toutes été remarquées par FALLERONI, mais pas baptisées par lui. Fait important : deux de ces espèces étaient toujours absentes des régions non impaludées ; l'une, *l'élutus*, que nous considérons pour plus de facilité, comme appartenant au groupe *maculipennis*, l'autre, le *labranchiae*. Chacun de ces types d'oeufs fut trouvé dans certaines parties de l'Europe à l'état de souche pure et il était clair, d'après les études des habitudes des anophèles de ces régions, que nous avions affaire à six races diverses de *maculipennis*. Celles-ci se différencient par certaines soies larvaires et la forme d'une épine de l'hypopygium, mais ces caractéristiques chavauchent à un tel point et sont si variables, qu'il ne nous est pas encore possible de distinguer les femelles ou les mâles d'une race, de ceux d'une autre race. L'indice maxillaire indiqué par ROUBAUD semble maintenant avoir une signification différente de celle qu'il proposait Sa valeur dépend de la race de l'insecte et non pas de la présence ou de l'absence d'animaux domestiques stabulés dans la région. La rivalité, si elle existe, se développe dans l'eau entre les larves et non dans l'air, entre les adultes. Ce pendant, ce fait costituerait encore une caractéristique importante, s'il se trouvait qu'il influençâ de quelque manière que ce fut les habitudes d'alimentation des races.

L'Italie semble être le seul pays dans lequel ces six races sont présentes. Parmi celles-ci, l'*atroparvus*, le *typicus* le *melanoon* et le *messeae* ne transmettent pas habituellement le paludisme. Cette maladie se limite donc dans ce pays à l'habitat de l'*elutus* et plus particulièrement du *labranchiae*, étant donné que le premier est plutôt rare en Italie. Il est curieux que l'*atroparvus*, qui est un des types inoffensifs en Italie, soit un vecteur du paludisme en Espagne, au Portugal et le seul transmetteur au nord des Alpes. La raison pour laquelle il transmet le paludisme dans une région et pas dans une autre, est un mystère que nous envisagerons dans ma prochaine conférence. Comme pour l'*elutus* et le *labranchiae*, ses gites à larves se trouvent dans les eaux saumâtres et, du fait que tous les vecteurs d'Europe possèdent cette caractéristique générale, le paludisme tend à suivre le littoral. Certaines exceptions ne font que confirmer cette règle, car alors qu'il existe des régions côtières (Viareggio, Naples) sans paludisme, on a remarqué que les gîtes à larves qui s'y trouvent contiennent de l'eau douce et, bien qu'il y ait des régions à l'intérieur des terres qui sont impaludées (Diamantina, Sicile), on observe souvent qu'elles sont caractérisées par des sources ou des lacs d'eau salée.

La distribution du paludisme en Europe coïncide par conséquent avec celle des races dangereuses du *maculipennis*, et si ce les-ci sont dangereuses, c'est parce qu'elles persistent à pénétrer dans les maisons et à piquer les habitants là même où les animaux domestiques sont très nombreux. Ainsi que nous disons que les races inoffensives sont détournées des habitations par les étables. Par conséquent, une carte de l'Europe indiquant la distribution du paludisme peut être aussi bien employée pour illustrer la distribution des races dangereuses de *maculipennis*. Cette distribution dépend des types de gîtes à larves observés dans chaque localité. En règle générale, le *messeae* préfère l'eau stagnante des grandes vallées fluviales de l'intérieur de l'Europe et le *melanoon* les rizières et les marais d'eau douce. Par contre, le *typicus* marque une préference pour les régions montagneuses arrosées de cours d'eau alimentés par des sources et c'est généralement la race des hautes terres. Les trois autres races préfèrent, ainsi que je l'ai déjà mentionné, l'eau légèrement salée des lacs et des marais du littoral, mais l'*atroparvus*, qui en tant que vecteur du paludisme constitue parfois une exception, en est également une, quant au choix de ses gites à larves dans certaines régions. En Espagne, par exemple, on le trouve sur le plateau central, se reproduisant dans de l'eau parfaitement douce, sans une teneur minérale élevée. Il existe ainsi de nombreux problèmes écologiques et épidémiologiques en relation avec les races, mais nous ne nous tromperons pas en affirmant que les régions de paludisme endémique d'Europe sont celles dans lesquelles les races dangereuses se sont solidement « retranchées », tandis que celles que constituent les « places fortes » des races inoffensives sont appelées régions à anophélisme sans paludisme ; cet état de choses a, par conséquent, très peu de rapport avec la prospérité de la population ou le niveau général de vie des individus. Il s'agit là d'un phénomène biologique lié aux différences d'habitudes alimentaires de variétés du *maculipennis* pas uniformement distribuées.

Position systématique des races.

Quelle est la position systématique de ces variétés ou races ? L'entomologiste répugne à accepter une nouvelle espèce qui n'est basée que sur des modèles d'oeufs ou sur le comportement des insectes. Les experts en génétique reconnaissent d'autre part des races biologiques, lorsqu'une espèce peut être divisée en groupes se recontrant dans la même localité et montrant des différences biologiques nettes, mais pour ainsi dire aucune anatomique. De tels groupes ne se croisent naturellement pas entre eux, sinon les différences ne se maintiendraient pas. Que se passe-t-il donc avec les groupes du *maculipennis* que l'on trouve souvent mélangés dans la même région ? Les mâles distinguent-ils les femelles qui nous paraissent si semblables et refusent-ils de s'unir à celles de la race étrangère ?

Ceci a été très difficile à établir, aussi bien dans la nature que dans le laboratoire ; dans la nature du fait que nous ne pouvons pas distinguer les femelles les unes des autres, et dans le laboratoire parce qu'une seule race s'accouple en captivité : l'*atroparvus*. Les entomologistes hollandais ont découvert néammoins un fait d'une grande importance pour la solution de ce problème : les *atroparvus* mâles peuvent fréquemment être amenés à s'accoupler en captivité avec des femelles d'autres races. Les resultats de ces croisements ont été du plus haut intérêt et des plus importants car dans aucun cas ils n'ont produit de génération F_1 (1ère filiation) fertile. Ces résultats ont été de deux sortes : ou bien aucune génération F_1 viable n'a pu être obtenue (comme c'est le cas par exemple avec les essais de croisement entre le buffle d'eau et la vache), ou bien les oeufs produisirent des hybrides bien portants mais anormaux au point de vue sexuel et, par conséquent, incapables de continuer la race (tel que c'est le cas avec les mules, qui sont généralement — mais pas toujours — stériles). La conclusion de ces observations est que dans les Pays-Bas où trois races sont mélangées, le croisement des microptères (*atroparvus*) avec les macroptères (*messeae* et *typicus*) est définitivement écarté, dans la nature par une barrière de stérilité mutuelle. Les mâles d'autres races refusent de coopérer aux expériences de croisement ; mais, étant donné qu'ils ne fécondent même pas en captivité les femelles de leur espèce il ne nous est pas possible de déterminer si ce fait provient d'une barrière naturelle entre les races ou de l'état de captivité incompatible avec l'accouplement des insectes eurygames. Néanmoins les résultats divers de la fécondation par l'*atroparvus*, révèlent parmi eux, de profondes différences. Ainsi l'*atroparvus* semble être particulièrement éloigné de l'*élutus*, à un moindre degré du *typicus* et du *melanoon* et se rapprocher le plus du *labranchiae*, bien que l'habitat de ce dernier ne coincide nulle part avec le sien.

Ces expériences ont eu comme résultat inattendu d'élever tout à coup l'*atroparvus* (et avec lui les autres formes) au rang logique de véritable espèces. La question de la stérilité mutuelle est, en effet, la preuve la plus sérieuse de la nature spécifique des différences entre les variétés. L'absence de croisement rend la valeur d'un groupe (d'après WALSH) égale à celle d'une espèce que l'on peut différencier grâce à des bases anatomiques, bien qu'il puisse être difficile de lui assigner une place définie en entomologie systématique. En d'autres termes, nous possédons maintenant une série de groupes mutuellement stériles dans une espèce qui a pu être, è un moment donné, homogène. Que nous les appelions variétés ou races, nous sommes en droit de les considérer comme des espèces. Ceci nous aide à comprendre le comportement différent des variétés de *maculipennis* en dépit de leur ressemblance physique extraordinaire. Nous comprenons immédiatemente la raison de dissemblances qui peuvent être si marquées dans leurs instincts et habitudes, leur hibernation, leurs gîtes à larves et leurs préférences pour certains hôtes. Ce sont ces différences qui de certaines races font des vectrices du paludisme et qui nous offrent peut-être la possibilité d'utiliser des méthodes biologiques pour interrompre la transmission du paludisme.

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RECENT ADDITIONS TO OUR KNOWLEDGE OF "ANOPHELES MACULIPENNIS" RACES

by

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I. Introduction.

The development of our knowledge of the races or varieties composing the species-complex *Anopheles maculipennis* was carefully summarised with a comprehensive bibliography by Hackett & Missiroli (1935). A brief guide or key to the varieties of *A. maculipennis* was also printed in a *Quarterly Bulletin* of the Health Organisation of the League of Nations, with photographs of the accepted egg-types and maps showing the distribution of the varieties as reported at that time. There is, therefore, no need in this introduction to do more than summarise briefly the status of our knowledge at the end of 1934.

Six different forms were then recognised on the basis of certain characters of the eggs—namely :

(*a*) The arrangement of the light and dark areas on the upper surface, often called the egg pattern or design, produced by the size and apical development of the papillæ of the exochorion, which in certain areas are reduced in size and height and are rounded on top so that they are transparent to incident light and allow the black chorion to show through, while in other areas they are tall, with exuberant, spreading, roughened tops irregularly stellate in shape and often coalescing with one another, very refractive to light and giving, in their maximum development, a hoar frost appearance to the lighter spots on the egg.

(*b*) The size of the float structure as indicated by the number of ribs and rugosity of the intercostal membrane, the floats being superficially described as being large or small, and rough (rugose) or smooth.

Upon carefully comparing the different forms of *A. maculipennis,* secured by breeding imagines under identical conditions from classified eggs with perfectly clear characters, other structural differences of a statistical kind have been found. The races differ in size, in the number of branches and the form of certain larval hairs, and in the form and number of the external spines on the claspette of the male hypopygium. These differences can only be determined by averaging a large number of cases, and do not serve to identify a given specimen.

It was found that the *atroparvus* males would not only mate with females of the same race in small cages, but would also inseminate females of all the other races, with viable results in certain cases. However, these experiments in cross-mating showed that *atroparvus* possesses a different genetic affinity for each of the other races, thus confirming the fact that these races differ genetically among themselves. For example, the product of the cross with *messeæ* is non-viable, whereas the cross with *labranchiæ* produces an F_1 generation with normal females and with males somewhat less than half of which possess well developed organs of reproduction. Crosses with the other races may give a healthy F_1 generation, but one or both sexes are sterile, as in the case of mules. According to this test, *atroparvus* is more remote from the races *messeæ* and *typicus* than it is from *elutus*, which is recognised as a different species. The cross with *elutus* gives non-viable larvæ which succumb, however, at an advanced stage.

The instincts, behaviour and physiology of the races show evident differences of such importance as to make malaria vectors of some and relatively harmless cattle-feeders of others.

II. RECENT OBSERVATIONS ON THE DIFFERENTIAL CHARACTERS.

Biometric Studies.

No character has yet been found which can be used to identify the adults of the various races except in the case of *A. elutus*. SWELLENGREBEL (1936) and his colleagues have reported that, in the absence of *typicus*, the larvæ of *messeæ* can be distinguished from those of *atroparvus* in the Netherlands by the type of palmate hair on the second segment, in conjunction with the number of branches of hair No. 2 on the fourth and fifth segments. The hibernating *messeæ* also differs from the semi-hibernating *atroparvus* by having rod-shaped salivary glands (DE BUCK & SWELLENGREBEL, 1935). SHUTE reports that the external harpaginal spine of the male hypopygium is more constant in shape and length than at first supposed and that it varies most in *messeæ*. He gives a key which differs somewhat from that published by HACKETT & MISSIROLI.

Table 1.

SHAPE OF EXTERNAL HARPAGINAL SPINE, *Maculipennis* RACES.

According to :

	SHUTE (1935)	HACKETT & MISSIROLI (1935)	HACKETT & LEWIS (1935) (Albanian specimens)
Banded eggs :			
Melanoon	—	Variable[1]	—
Messeæ	Variable	Variable	Variable
Subalpinus	—	—	Almost always bluntly pointed or rounded
Typicus	Blunt or round	Blunt or round	Blunt or round
Eggs without bands :			
Atroparvus	Always sharp and tapering	Always pointed	—
Labranchiæ	Always sharp and tapering	Always pointed	—
Elutus	Blunt or rounded	Variable	—

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[1] 50% pointed.

It has been the custom, in biometric studies of larval hairs and hypopygial spines, to consider each side of the specimen as a separate case, in view of the lack of symmetry often displayed in these bilateral characters. This leads to the somewhat paradoxical situation that one half of a larva or a hypopygium may lend weight to one classification and the other half to a different one altogether, although the specimen itself may be undoubtedly of pure race and not a hybrid. Furthermore, in the case of the number of branches of hair No. 2 on the fourth and fifth segments, an analysis of a large number of observations shows that the variation between the two sides of the same larval segments is less than that between one segment and the next, or between one individual and another of the same race. Hence, taking the two sides as separate cases does not really double the basis of computation, since there is a high correlation between them. A statistical analysis shows that each larva should be considered a single case, and that with a sufficient number of specimens, the total number of branches of any two hairs may be taken in practice to represent the larva, provided they are located on different segments. It is probable that the same situation of bilateral correlation exists in the male hypopygium as well and that both sides should be taken into account, in tabulating specimens, as regards the bilateral symmetry and form of the external spines.

Martini (1933) suggests that the shape of the spine on the dorsal (external) lobe of the claspette may be a function of its position. A twin to the external spine is most like it if the two are side by side springing from the same level. The external spine in *messeæ* is most apt to be blunt at the most dorsal position. The tendency to produce blunt spines decreases from dorsal ventrad.

Egg Characters.

The characters of the egg still constitute the only known method of identifying individuals. Very little confusion has developed in nomenclature. One or two authors continue to employ the original classification of Falleroni and confuse *atroparvus* with *labranchiæ*. Clear-cut genetic differences have long confirmed the validity of their separation into distinct races, while the eggs themselves have only a superficial resemblance. The brownish *atroparvus* egg is slender with smooth float membranes, while *labranchiæ* lays a broad egg with very small and very rugose floats. *Atroparvus* is found more and more to be an inland breeder as well as a coastal form, and is now reported as far south as Naples.

It has been discovered that the egg characters are influenced by temperature, and also show variations between different geographical regions.

(a) *Temperature.*—The early spring eggs, especially those obtained from the generation issuing from hibernation, present the most difficulties in classification. Unpublished observations by D. J. Lewis in Albania and by M. A. Barber in Greece point to the influence of low temperatures on the egg or adult female as the main cause of atypical egg batches in spring. Barber stated in a personal communication to the author in May 1935 :

> " A dark, almost black egg with bands scarcely defined, grown from egg to adult under higher temperatures, will give a mosquito which lays bright *typicus* eggs with very sharply defined bands. "

R. B. Hill writes from Portugal :

> " From one batch of plain dark grey eggs without a trace of pattern the resulting females laid typical *atroparvus* patterned eggs."

Rivera & Hill (1935) also mention that eggs are lighter in colour and smaller at the height of summer. In Albania in midsummer, Lewis obtained darker eggs experimentally in *typicus* and *subalpinus* by subjecting females to temperatures of about 16°C. between ovipositions. The development of the papillæ on the exochorion is apparently retarded at low temperatures, the apical roughening is less exuberant, and the whole appearance of the egg is darker, due to increased translucency of the outer coat.

Corradetti (1934) states that the egg-pattern is a Mendelian character, and similar observations have been made before with regard to other insects. For example, Jennings (1930) mentions that the race of silkworms with brown eggs differs from the race with slate-coloured eggs in one gene. When the eggs of the brown race are fecundated by slate-coloured males, the resulting eggs are slate and successive generations (since slate is dominant) follow the simple Mendelian rule. However, *labranchiæ* females inseminated by *atroparvus* males laid *labranchiæ* eggs, since the characters of the anopheline egg are already fixed before its fertilisation from the spermotheca. The eggs laid by the first hybrid generation were mixed, following the Mendelian rule, not only as to pattern, but also in shape, and in the size and rugosity of the floats. All the eggs of a single clutch were alike.

Rivera & Hill (1935) studied the variations in *atroparvus* through six inbred generations derived from a single female. The variations were not significant in pattern of eggs or rugosity of float membrane. The eggs became somewhat lighter in colour as summer advanced, while the floats were never rough, 4 to 8% showing occasional rugosities. There was no variation in the palmate hair of the second segment, the leaflets being always narrow with little pigment, while the branches of hair No. 2 on the fourth and fifth segments never average more than 4.5. The wing length diminished from 5.2 in the original (spring) female to 4.6 in the sixth (summer) generation, and the maxillary index also decreased with fair regularity from 16.5 in the original female of the spring generation to 14.3 in the last (summer) generation.

(b) *Geographical differences.*—Certain variations are found between one region and another, in eggs presumably of the same race. It is these which tempt observers to make subdivisions of known races, and in certain instances to create new race-names. Several new races have been proposed in addition to the original six :

A. maculipennis var. *fallax* Roubaud, 1934—from Normandy, France. Differs from *atroparvus* in having larger floats and a branched hair instead of an intermediate palmate on the second segment of the larva. Hair No. 2 (fourth and fifth segments) averages more than 4.5 branches. The external harpaginal spine is blunt as in *messeæ* and *typicus*. It is found breeding in fresh water, and has a lower salt tolerance than *atroparvus* (Roubaud, Colas-Belcour, Treillard, 1935). It differs from *messeæ* in the absence of bands on the egg and rugosities on the floats ; the race is stenogamic, and reproduces indefinitely in captivity. It crosses easily with *typicus* from the Netherlands. It is zoophilous and multidentate.

A. maculipennis var. *sicaulti* Roubaud, 1935—from Rabat, Morocco. Allied to *labranchiæ*. Differs in having a narrower white spot on the wing fringe. Branches of larval hair No. 1, second segment, filiform instead of palmate. Eggs more obtuse, darker, but with fewer markings than *labranchiæ*.

A. maculipennis var. *pergusæ* Missiroli, 1935—from Sicily, Italy. Allied to *labranchiæ*. Differs in having broader, shorter eggs almost without markings. Larval and adult characters not described.

[*Note.*—SERGENT, Et. (1935), has described a form from Algiers intermediate between *labranchiæ* and *sicaulti*. It is evident that *labranchiæ*, like its nearest relative *atroparvus*, shows a number of geographical variations and modifications. Being eurygamic, the genetic affinities between these nearly related forms cannot yet be ascertained, and no attempt has been made to test them in crosses with *atroparvus* males. Their status as new varieties remains to be proved.]

A. maculipennis var. *subalpinus* Hackett & Lewis, 1935—from Albania. Belongs to the barred-egg group. Differs from *messeæ* in having eggs with smooth float membranes and fewer ribs; more branches to larval hair No. 2 on fourth and fifth segments, and invariably a branched hair instead of a palmate on the second segment. The external harpaginal spine is prevailingly blunt and double as in *typicus*. It breeds in fresh-water marshes and is found in the northern Mediterranean basin from Spain to the Balkans, particularly in the uplands.

[*Note.*—The egg of this variety was first classified as *messeæ* until it was found to have smooth floats, and then as *melanoon*, the description of which was expanded to include both black and banded eggs. It is now proposed to restrict the name *melanoon* to black eggs with smooth floats, and *subalpinus* to banded eggs with smooth floats.]

The American forms of *A. maculipennis* have been described recently by HOFFMANN (1936), DAMPF (1935) and others. The American races are distinguished by the presence or absence of the coppery spot on the wing fringe. According to HOFFMANN, the race with the apical bronze spot should retain the name *occidentalis* (DYAR & KNAB), and its range is given as extending from California to Alaska, across Canada and into Maine and Massachusetts.

The race without the spot is found in high areas in the southern United States and Mexico and has been named by Hoffmann *aztecus*. This requires further study, for Freeborn wrote in 1926 :

> " In the valleys and foothills of the Sierra (California) the spot is almost always impossible to distinguish."

The females which I have seen in California did not have any spot on the wing fringe. No egg, larval or hypopygial characters have been found to distinguish the races. The eggs which I examined were a uniform grey with rather small, smooth fan-shaped floats of about twelve ribs. On the other hand, Hoffmann (1935) describes the Mexican eggs as having large, rough floats with twenty-five ribs.

It is not unlikely that new local varieties will continue to be found over the vast range of *A. maculipennis*. The reporting, however, of a new race or variety should take into account the special characters which have been found to be useful in separating the races of this species. Hence such reports should pay particular attention to certain points not always included in ordinary descriptions. The following form may be useful as a reminder of these details which unfortunately are lacking in reports of supposedly new races omitted from the above list on account of insufficient data. Our information with regard to the American forms is exceedingly incomplete.

Name : Where and when found ; range.

Characters :

Eggs : Number of batches seen—upper surface (banded or not, dark areas, etc.). Floats : number of eggs examined—date—intercostal membrane smooth or rugose.

Average number of ribs—standard deviation—maximum number, minimum number.

Larvæ : 2nd segment, hair No. 1 : number examined—filiform, intermediate or palmate. 4th and 5th segment, hair No. 2 : number examined—average number of branches—standard deviation—maximum number of branches, minimum number of branches.

External harpaginal spine :
Percentage with spines on both sides rounded, both pointed, and mixed. Percentage single—double—triple.

Bionomics : Larval habitat : water body type :
Depth—permanent or temporary—movement— temperature—salt content—shade—vegetation (submerged, vertical, etc.)—upland or lowland—distance from sea—breeding season.

Habits : Winter behaviour—mating in captivity—crossbreeding.

III. MATING IN CAPTIVITY.

HACKETT & BATES (1936), in Albania, have obtained swarming of males in captive groups of *A. elutus* and of the race *typicus*, first in a very large outdoor cage (10.5 × 5 × 6.2 metres) and later in a smaller laboratory cage (55 × 55 × 110 centimetres). Fertile eggs were obtained, and it is hoped that permanent colonies may be established and kept under observation.

IV. GEOGRAPHICAL DISTRIBUTION OF THE RACES.

As for the geographical distribution of the accepted forms, egg-surveys have been made in regions hitherto unreported :

(1) *Sweden* (EKBLOM, 1935, with map). *Messeæ* is generally distributed and with it is associated *typicus*, except in the extreme south, where it occurs together with *atroparvus* (which the author continues to call *labranchiæ*, after FALLERONI). The three together are found only on the west coast.

(2) *Yugoslavia* (ZOTTA, 1935). The usual Balkan forms are found—to wit, *messeæ*, *typicus* and, on the coast, *elutus*. In addition, it is stated that *labranchiæ* occurs on the northern Dalmatian coast, and that an atypical egg with bars like *messeæ*, but with a highly contrasted black and white pattern, is found in the uplands. In a private communication, the author agrees that this is probably *subalpinus*.

(3) *North Africa* (Algiers : Sergent, Et. & Trensz, 1935 ; Tunis : Galliard, 1935, Villain *et al.* 1935 ; Morocco : Roubaud, 1935). The only races are *labranchiæ* and the related form *sicaulti.* Galliard says that " it is certain that *elutus* must exist in the coastal region ", but this has never been confirmed.

(4) *Syria* (unpublished report by Kligler, 1935). No member of the *maculipennis* group was found except *elutus*, which was breeding in fresh-water streams and marshes. None was found in a coastal marsh near Alexandretta, and none is mentioned as breeding in brackish water.

In the Union of Soviet Socialist Republics, there has been no survey since that of Hackett & Barber (1935), but occasional reports from scattered regions indicate that only *messeæ* and *typicus* are found in the interior, while *elutus (sacharovi)* occurs on the Caspian and *atroparvus* on the Black Sea near Odessa.[1]

Missiroli (1935) suggests that inconstancy of temperature is an important factor in larval ecology and may help to explain the distribution of the races and species. Thus while *plumbeus* is adapted to constant thermic conditions at 15° or 16°C., and so probably is *bifurcatus*, *typicus* prefers a temperature oscillating rather widely between day and night.

V. Systematic Position.

The word race is still used more frequently than variety, since the genetic and biometric differences between the forms are more striking than the structural. The name *subspecies* has fallen out of use recently, although in their mutual sterility the forms resemble true species. In spite of the difficulties which systematic entomologists find in dealing with the *maculipennis* complex, the distinctions involved have assumed enormous importance in malariology. This is only partly because, in Europe, races like *labranchiæ* and *typicus*, though indistinguishable under the microscope, frequent in many regions a different range of host, making one a dangerous anopheline and the other a non-vector, and, being adapted to different larval habitats, permit the economies of race-control. It is also important because most of the widespread anopheline species of the world have been found on examination to be complexes of this sort. SWELLENGREBEL, in an as yet unpublished report, describes the fresh and salt water forms of *A. ludlowi*, each of which is divided into vectors and non-vectors of malaria. *Hyrcanus, barbirostris* and *funestus* of the Old World and *pseudopunctipennis* and *tarsimaculatus* of the New are examples of species whose biological characters and relationship to malaria differ from place to place. According to DE MEILLON (1935), the *A. funestus* female, which frequents dwellings in South Africa, and that of *A. leesoni*, which does not, can only be told apart by their eggs.

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[1] See *Medical Parasitology*, 1934, **3**, Nos. 4, 5 and 6.

DIEMER and VAN THIEL (1936) suggest that *A. maculipennis* consists of only three fundamental biotypes, each of which has spread over the entire range of the species and has become adapted in different geographical regions to local conditions, forming a group of non-overlapping forms clearly distinguishable from one another, yet closely related and with many common characters. For example, *atroparvus-labranchiæ-elutus* form such a group or (to use the expression of the authors) *geobiotype*, which divide all of Europe between them, have a similar toleration for salt water, lay non-banded eggs, are more closely akin by genetic tests to each other than to the rest, and possess in common a wide range of hosts on which they feed, making them malaria vectors even in the presence of stabled domestic animals. The authors suggest that *messeæ-melanoon* may form a second geobiotype, for they may not overlap as much as is now believed, while *typicus* stands alone as a third.

This attractive hypothesis gains support from observations of others which the authors have not taken into account. The setting-up of a new race, *subalpinus*, may resolve the difficulty arising from the overlapping between *messeæ* and *melanoon*, with both of which it has been confused and between which it might well serve as a buffer. As for *typicus*, HACKETT & BARBER (1935) found a distinct type in the Caucasus with minute floats, and it is not unlikely that it will eventually be divided up into geographical sub-races. According to this theory of DIEMER & VAN THIEL, one would be apt to find only three *maculipennis* races mingling in any one region, and this, in fact, is fairly in accordance with our present rather deficient knowledge of their distribution. North of the Alps we find *atroparvus, messeæ* and *typicus*, in the Iberian Peninsula *atroparvus, subalpinus* and *typicus*, in Southern Italy *labranchiæ, melanoon* and *typicus*, and in the Balkans *elutus, subalpinus* and *typicus*. The presence of two members of the same group in the same locality has been frequently reported, but either one of the two is relatively uncommon, or there may easily have occurred errors in classification.

VI. RELATION OF THE RACES TO MALARIA.

Virtually all the malaria of Western and Southern Europe due to *A. maculipennis* is transmitted by *atroparvus, labranchiæ* and *elutus*. In East Macedonia, where *messeæ, typicus* and *elutus* occur together, BARBER & RICE (1935) dissected about 37,000 specimens in 1932, 1933 and 1934. The results were as follows :

	Number dissected	Percentage infected
Elutus	22,200	1.29
Messeæ and *typicus*	14,713	0.07

Thus, nineteen infected *elutus* were found for every infected *maculipennis*. " *Maculipennis (messeæ, typicus)* in our region ", write the authors, " where domestic animals are plentiful, is probably of little or no practical importance in the transmission of malaria." Similarly, SWELLENGREBEL and his colleagues (DE BUCK, SCHOUTE & KRAAN, 1936) dissected *atroparvus* and *messeæ* taken together in the same houses in a malarious village in the Netherlands.

	Number dissected	Percentage infected
Atroparvus	26,521	7.73
Messeæ	1,223	0.0008

Only one infected *messeæ* was discovered in all.

But the early hope that the fresh-water races, *messeæ, melanoon, subalpinus* and *typicus*, might turn out to be non-vectors of malaria everywhere has not been fulfilled. For example, the malaria of the Volga basin, that of the Danube above the delta, and that of Central Yugoslavia, is undoubtedly due to *messeæ* or *typicus* or both. *Melanoon* and *subalpinus* have not as yet been incriminated. This malaria may be due to the existence of less zoophilic sub-races of *messeæ* and *typicus*, or, what is more likely, to the fact that social and agricultural conditions do not yet provide in parts of Eastern Europe a sufficient barrier of stabled domestic animals to protect human beings from dangerous contact with zoophilic anophelines. The three factors : number of children, number of anophelines and number of animals, are subject to different values and combinations which seem to determine the level of transmission in the presence of the zoophilic races. The present policy of the Soviet Government to increase as rapidly as possible the number of domestic animals in villages in the Volga basin should permit the local malariologists to evaluate the influence of these factors. On the whole, the moderate intensity and prevailingly tertian type of malaria in these regions must be fairly comparable to that of Western Europe a century ago, when conditions still permitted malaria to maintain itself, with the help of anopheline races now innocuous.

There remains the mystery of *atroparvus.* In Northern Europe, it maintains a mild endemic of *P. vivax* malaria in the Province of North Holland, in the German area around Emden and possibly on the English east coast, but nowhere else. South of the Alps it is harmless on the Mediterranean shores of Italy, France and Spain ; it maintains an intense endemic malaria (of both *vivax* and *falciparum* infections) on the Atlantic coast of Portugal and in Western Spain. In the Netherlands, Swellengrebel and his colleagues believe that *atroparvus* females do not become infected to any extent until after August 15th, when they cease their sexual activities and seek winter quarters for semi-hibernation in dwellings and stables. Martini (1934) is not convinced that this is true in general for all the malaria transmitted by *atroparvus* in Northern Europe. His observations indicate that summer infections cannot be excluded in Germany. He also found in records made between 1860 and 1870 that many summer visitors from Leipzig used to be infected with malaria during July and August on the island of Norderney in the North Sea not far from the Dutch border. It is his opinion (personal communication) that the most dangerous period began in mid-July, before anopheline hibernation set in.

This does not explain why *atroparvus* is not a vector in the rest of Northern Europe. It is possible that the reason is to be sought in the quantitative relationships between *atroparvus* densities, methods and extent of animal husbandry and number of children

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SWARMING OF THE MALES OF CERTAIN EUROPEAN ANOPHELINES IN CAPTIVITY

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Swarming of the Males of Certain European Anophelines in Captivity

THE study of the biology of the European *Anopheles*, particularly the complex of forms known as *Anopheles maculipennis*, has been greatly hampered by the difficulty of inducing mating under laboratory conditions. Missiroli and I[1] have given a summary of the experiments made with these mosquitoes, of which only the 'race' known as *atroparvus* has mated in captivity. In this form, it appears that there is no sexual dance on the part of the males as a preliminary to mating. Curiously enough, females of all the races will mate with *atroparvus* males, but repeated attempts on the part of many investigators to get them to pair with males of their own kind have failed.

Some years ago, I constructed a large cage of wire netting, 10·5 metres long, 5 metres wide, and 6·2 metres high, in connexion with the malaria laboratory in Albania. The cage was designed for the observation of the behaviour of *maculipennis*, and an attempt was made to make the conditions within the enclosed area as natural as possible. A cement pool, a stable, a small laboratory, and a tree were included within the cage. Fertile *typicus* eggs were found in the pool in 1934, but in small numbers compared with the quantity of mosquitoes released, and no sexual activity was observed in the cage. Between June 19 and July 12, 1936, we released in the cage 4,500 adults of *typicus* bred from the egg, and on July 7 we first observed swarming of the males, although larvæ had been found in the pool some days previously. Since then we have found the males swarming almost every evening, the swarm usually starting between 7.15 and 7.30 p.m., and the principal swarm always forming in the same position: about half a metre beneath the top of the cage, directly under one of the cross beams, and about half a metre from one of the upright poles supporting the side. This orientation, under the cross beam, seems very curious, and we should consider it to be fortuitous were it not repeated so frequently.

The wire cage is, of course, completely open to external stimuli, and we have been carrying on some experiments in the opposite direction: in a room insulated so far as possible from external stimuli, so that all the reactions of the mosquitoes would be to factors within the room. The room used is 2·5 metres long, 2·3 metres wide and 2·6 metres high. It is constructed of mud brick, with an air space between the ceiling and the roof; the windows are kept closed and have been covered on the outside with canvas curtains, so that the maximum light during the day is only about five foot-candles. The climate within the room remains remarkably constant, the temperature showing a diurnal fluctuation of about 5° around a mean of 20°, the humidity varying between 84 and 94 per cent. We released in this room about a thousand *maculipennis* adults bred from *typicus* eggs, but we did not observe any sexual activity, and we only obtained two batches of fertile F_2 eggs. At the same time, about 600 *Anopheles superpictus* were released in the room, and the males of this species were found to swarm under these conditions very readily, and numerous fertile *superpictus* eggs were found in the aquarium in the room. Up to July 27 we had released in the same room about 500 *Anopheles elutus*, raised in the laboratory. The males were first observed swarming on July 28, and afterwards batches of fertile eggs were found in the aquarium.

We are thus able to observe under varying conditions the sexual activities of the three malaria vectors of Europe. A few *bifurcatus* were also released in this room, and one evening four or five males were observed forming a swarm, and fertile eggs of this species were afterwards obtained.

L. W. HACKETT.
MARSTON BATES.

Seksioni Antimalarik,
Tirana, Albania.
August 1.

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[1] *Rivista di Malariologia*, 14, 45 (1935).

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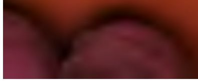

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XLIII.—NOTE ON THE ARRANGEMENT OF THE MESENTERIES IN THE PARASITIC LARVA OF *HALCAMPA CHRYSANTHELLUM* (Peach). BY A. C. HADDON, M.A., M.R.I.A., Professor of Zoology in the Royal College of Science, Dublin. (Plate XI.)

[Read, February 16, 1887.]

IN 1859 L. Agassiz recorded from the east coast of North America an Actinia parasitic on Medusæ, which he named *Bicidium parasitica.* This has since been found by Verrill in 1862, and by A. Agassiz in 1865. Still more recently (1884), Mark[1] has given a preliminary account of a larval Edwardsia, which is parasitic within the gastro-vascular canals of the Ctenophore *Mnemiopsis leidyi.*

On this side of the Atlantic, T. Strethill Wright, in 1859, gave an account of a small Actinia, also parasitic, on Hydromedusæ, from the Firth of Forth, which he named *Halcampa Fultoni;* and, in the following year, F. Müller described a similar form, which he named *Philomedusa vogtii*, from the Santa Catherina, on the Italian Riviera. E. Graeffe described, in 1883, a parasitic Halcampa from the Adriatic, which, "as the development of *Halcampa chrysanthellum* is not known, this form must, provisionally, be separated from *H. chrysanthellum* as *H. medusophila.*"

The author exhibited, and made remarks upon, two specimens of a parasitic Halcampa at a meeting of the Royal Irish Academy, on June 22, 1885, and a record was published in the following year. In this communication it is stated that Prof. A. Macalister of Cambridge (late of Dublin) had informed the author, by letter, that he had met with this Halcampa, and perhaps another form, but neither of them in Dublin Bay. Specimens were also obtained in Dublin Bay in June, 1886, and on June 6, in the same year, off

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[1] "Selections from Embryological Monographs," compiled by A. Agassiz, W. Faxon, and E. L. Mark, Bull. Mus. Comp. Zool., Harvard Coll. (Camb., U. S. A.), p. 43, pl. xii.

Ballycotton, Co. Cork. On the same day *Halcampa chrysanthellum* was dredged from fifty-two fathoms.

Bibliography of Larval Actiniæ Parasitic on Medusæ.

North European Seas.

Peachia fultoni, . .	T. Strethill Wright, 1859, *Proc. Roy. Soc. Edinb.*, ii., p. 91, 1860 ; *New Edinb. Phil. Journ.*, xii., p. 156.
Halcampa, ,, . .	Reprinted in *Ann. Mag. Nat. Hist.* (3), viii., 1861, p. 132.
,, ,, . .	G. Leslie and W. A. Herdman, 1881, *Invert. Fauna of Firth of Forth*, p. 63 (merely repeats Wright's record).
Philomedusa ,, . .	A. Andres, 1883, *Le Attinie, Atti. Acc. Rom.* (3 A). xiv. [*Fauna, u. Fl. d. Golfes v. Neapel* (1884), p. 114.]
Halcampa chrysanthellum,	A. C. Haddon, 1886, *Proc. Roy. Dublin Soc.* (N. S.), v., p. 11 ; *Proc. Roy. Irish Acad.* (2), iv., Sci., p. 527. (Noted in *Zoologist*, 1886, p. 7).

There can be little doubt concerning the justice of considering the above to be the larval form of *Halcampa chrysanthellum ;* the form, colour, structure, and histology support this conclusion.

In the only three localities where the parasitic larva has been hitherto found the adult *Halcampa chrysanthellum* has also been obtained, viz. Firth of Forth (Leslie and Herdman, *loc. cit.*, p. 62, on the authority of F. E. Schulze, "Zoologische Ergebnisse der Nordseefahrt," III. Coelenterata, p. 140: Berlin, 1874), Dublin Bay, and Cork (A. C. H.).

In a former paper [*Proc. Roy. Dubl. Soc.* (N.S.), v., 1886, p. 1] I have endeavoured to show that the forms known as *Edwardsia duodecimcirrata*, Sars (from Norway and E. Denmark), *Zanthiopus bilateralis*, Kef., and *X. vittatus*, Kef. (from N. France), are one and the same with this species. If this be so, the parasitic larva must have an equally North European range.

Mediterranean.

Philomedusa vogtii, . .	Fritz Müller, 1860, *Wiegmann's Archiv f. Naturg.*, xxvi., p. 57 [reprinted in *Ann. Mag. Nat. Hist.* (3), vi., 1860, p. 432.]
„ „ . .	A. Andres, 1883, *Le Attinie*, *Atti Acc. Rom.* (3 A), xiv. [*Fauna u. Fl. d. Golfes v. Neapel* (1884), p. 112].
Halcampa medusophila, .	E. Graeffe. 1883, *Boll. d. Soc. Adriatica di Sci. Nat. Trieste*, vii.

As *Halcampella endromitata* (Andres), is the only Mediterranean example of the Halcampidæ, the above-mentioned forms are probably the parasitic larva of that species.

Coast of New England—North-East America.

Bicidium parasiticum, .	A. Agassiz, 1859, *Proc. Boston Soc. Nat. Hist.*, vii. (1861), p. 24.
„ „ .	A. E. Verrill, 1862, *Mem. Boston Soc. Nat. Hist.*, i. (1866), p. 31, pl. i., figs. 14, 15.
„ „ .	E. C. and A. Agassiz, 1865, *Seaside Studies in Natural History, Boston*, p. 15, fig. 14.
Peachia parasitica, . .	A. E. Verrill, 1866, *Proc. Boston Soc. Nat. Hist.*, x., p, 338.
„ „ . .	A. E. Verrill, 1873, *Report U. S. Fish Com.*, i., 1871–2, p. 739.
Philomedusa „ . .	A. Andres, 1883, *Le Attinie*, *Atti. R. Acc. Lincei, Rome* (3 A), xiv. (*Fauna u. Fl. d. Golfes v. Neapel*, 1884, p. 112, fig. 9.

From Verrill's accounts (1862 and 1866) there can be no doubt that the above parasitic Anemone is really a Peachia; it must, therefore, be known, for the present, as *P. parasitica*; but in the latter paper Verrill states that it is very much like *Siphonactinia* (*Peachia*) *Bœckii* (Dan. & Kor. 1856) in form and colour. The colour is purplish-brown, or red, and the length 32 mm. – to 46 mm. With the exception of two specimens of very large size found buried in the gravel, at low water-mark, at Eastport, Maine (Verrill, 1873), this form is only known as parasitic in the lip-folds of *Cyanea arctica*, from Cape Cod to the Bay of Fundy.

Southern Ocean.

Actinia clavus, . .	Quoy et Gaimard, 1833, *Voyage de l'Astrolabe*, p. 150, pl. x., figs. 6, 11.
Ilyanthos „ . .	Milne Edwards, 1857, *Hist. Nat. des Coralliaires*, i., p. 284.
Philomedusa clavus, . .	Andres, 1883, *Le Attinie, Atti. Acc. Rom.* (8 A) xiv. [*Fauna u. Fl. d. Golfes v. Neapel* (1884), p. 114.]
Halcampa „ . .	R. Hertwig, 1882, *Actiniaria*, "*Challenger*" *Reports*, p. 92.

Quoy and Gaimard found several specimens of this Halcampa entangled (*engagés*) in the tentacles of a medusa. It was 7–8 lines long in its greatest extension, and only three when contracted; translucid white in colour; 12 short tentacles. They obtained it in Bass' Straits, Australia, lat. 38° S.

R. Hertwig identifies an Halcampa dredged by the *Challenger* at Kerguelen (25–120 fathoms) as this species.

It is interesting to observe that certain (at least) of the members of the three families, Edwardsidæ, Halcampidæ, and Siphonactinidæ, pass through a stage during which they are parasitic on Medusæ or Ctenophores. There is now a good deal of evidence in favour of the view, that the Edwardsidæ and Halcampidæ are more closely related than was formerly thought to be the case; and, so far as my investigations on Peachia have gone, I am led to believe that the Siphonactinidæ are closely related to the latter. Be this as it may, the genus Philomedusa must now be discarded.

As before mentioned, in 1885 I found one or two specimens of the larval Halcampa in Dublin Bay, and again in 1886, in July of that year, I also found a specimen off the coast of Cork. They were usually attached to the stomach on the sub-umbrella (Pl. XI., figs. 1, 2) of different species of Leptomedusæ. Occasionally they adhered to the margin of the disc. With a little care they can be kept alive some time, and will feed on small pieces of meat when medusæ are not to be had.

When first obtained some specimens measured a little under 3 mm. in length, and one grew to about 5 mm. in length.

The body was sub-conical in form, the column not being distinctly divided into the three regions (capitulum, scapus, and physa) so characteristic of the adult. The middle portion was especially corrugated, and indented at the insertion of the mesenteries. The body could be slowly lengthened or contracted; it was uniformly clothed with small cilia. There were only eight short tentacles. At first they were very short, but afterwards they grew relatively longer.

The Medusa appears to be but little incommoded by the parasite; but it probably succumbs in time to its guests. In its ordinary condition the Anemone sinks in the water when taken from the Medusa; but it can extrude its mesenteries through its mouth for a considerable distance (Pl. XI, fig. 5). These enable it to float at the surface of the water, and, at the same time, to attach itself to passing Medusæ. This is probably the manner by which it secures a continual supply of food.

They had a uniform yellowish flesh-colour, with eight rudimentary tentacles. The tentacles grew longer, and were tinged with brown and yellowish white. The disc also became variegated with brown, and the body translucent, revealing the yellow œsophagus. At the last observed stage the body was almost colourless—the œsophagus yellow, the capitulum possessed a pair of cream-coloured spots below each tentacle, and the insertion of the mesenteries were of the same colour—the eight tentacles had on their oral surface two transverse bars of white at the base, and a single bar half-way along their length. Above this was a large brown spot, and a pair below it; and above the basal lines, between the two brown spots, is a small white one. The disc was prominent, with white radial lines, the areas being brown, finally speckled with white, each having prominent white spots at the mouth.

Although there were eight tentacles there were twelve mesenteries. The tentacles were arranged in two groups of three, and a

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single tentacle between each group. A deep siphonoglyphe, was present, thus causing the mouth to be T shaped. The siphonoglyphe, being in the axial line, indicates the disposition of the tentacles. On reference to Pl. XI., fig. 4, it will be seen that the intermesenterial chamber on each side of the axial or directive chamber is produced into a tentacle. Of the three remaining lateral chambers, only the centre possesses a tentacle.

All the previous accounts of the parasitic larva of Halcampa agree in the fact of twelve tentacles being present. This can only be accounted for by supposing that the larvæ were more developed than mine. This was certainly the case in Strethill Wright's specimens, and in my oldest examples I found indications of the sprouting of some of the missing tentacles. It is, of course, possible that the Mediterranean form acquires its twelve tentacles very early.

Meyer and Möbius (Arch. f. Naturg., 1863, p. 70) mention that in their adult examples of "*Edwardsia duodecimcirrata*," Sars. [*Halcampa chrysanthellum*], the number of tentacles varied from eight to twelve, but never more than the latter number.

By making a series of transverse sections I was enabled to trace out the arrangement of the mesenteries in a more satisfactory manner than could be effected by an examination of the living animal.

In the œsophageal region, the twelve mesenteries appear to have equal importance. The siphonoglyphe causes what may be termed the ventral directive mesenteries to be much bent. At the lower extremity of the œsophagus four of the mesenteries fall short of joining the œsophagus. The siphonoglyphe extends for a short distance beyond the œsophagus proper (Pl. XI., fig. 8).

In the gastric region of the body there are eight large mesenteries, which alone bear the swollen digestive borders. It will be noticed that it is those intra-mesenterial chambers, bounded by a strong and a weak mesentery, which are not prolonged into tentacles. The dorsal directive mesenteries also appeared somewhat smaller than the remaining six. The same general arrangement occurred at the posterior end of the body, except, of course, that the mesenteries have no thickened edges.

It is probable that at a slightly earlier stage only the eight strong mesenteries are present, as an increase in the number of tentacles with the growth of the animal is characteristic of most sea-anemones, and in our species the adult has twelve rudimentary mesenteries in addition to the twelve primaries [cf. *Proc. Roy. Dub. Soc.* (N.S.) v., 1886, p. 12, fig. 4]. The same occurs in *H. arenacea*, Haddon[1]; but according to R. Hertwig, there are only the twelve primaries in *H. clavus*, Quoy et Gaimard.

The brothers Hertwig[1] first insisted upon the systematic importance of the disposition of the muscular bands on the mesenteries. A comparison of the diagrams on Pl. XI. will demonstrate the fact that the eight strong mesenteries of the larval Halcampa perfectly corresponds with the eight mesenteries of Edwardsia. The Hertwigs have further shown that the normal Hexactina pass through a stage in which there are eight strong and four weak mesenteries (Pl. XI., figs. 10, 11); but it will be seen that these mesenteries do not correspond with those of the larval Halcampa and adult Edwardsia on the one hand, or with those of the Octactiniæ on the other.

The inequality in the development of the septa of the adult Halcampa was first pointed out by R. Hertwig[2] (Actiniaria, "*Challenger*" *Reports*, Zoology, vi., 1882, p. 95). He found that four were somewhat smaller than the eight others. I have quoted (*loc. cit.* pp. 7, 8, footnote) an observation of Dixon's confirming this, and Strethill Wright found the same in his larval form. He says:— "Eight septa were continued downwards to the lower extremity of the body, and had their free edges bordered by a convoluted ciliated band, furnished with cnidæ, or thread cells; the intersepta (*i.e.* the four smaller mesenteries) bore no convoluted bands."

On a future occasion I propose to give a detailed account of the anatomy of *Halcampa chrysanthellum;* for the present I would merely state that I find that, in the adult, the generative organs only occur on six mesenteries. These correspond with the eight strong mesenteries mentioned above, less the dorsal pair. The axial, or directive mesenteries, which support the siphonoglyphe, are here considered as the ventral, and the opposite pair as the dorsal.

The Hertwigs also pointed out that the Actinidæ (larval forms)

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[1] First Report "On the Marine Fauna of the South-west of Ireland—Actinozoa," *Proc. R. Irish Acad.* (2) iv. (Sci.), 1886, p. 616.

[2] Die Actinien (Studien zur Blättertheorie), O. and R. Hertwig, Jena, 1879.

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Edwardsiæ, and Alcyonaria exhibit three different ways in which the eight mesenteries may be disposed. They regarded the mesenteries as symmetrical—*i.e.* four dorsal and four ventral in the Actinidæ, as six dorsal and two ventral in the Edwardsiæ, while in the Alcyonaria all the eight mesenteries are dorsal.

Although my observations are incomplete, I have thought it desirable to place them on record, as it may be some time before I am able to discuss the question at greater length. For the present, we may assert that, although the adult Halcampa closely resembles the ordinary Actiniæ in the ratio of its tentacles, and the disposition of its mesenteries, the larval form is undoubtedly more nearly related to the Edwardsiæ.

EXPLANATION OF PLATE XI.

(*Figs.* 6-14 *are purely diagrammatic.*)

Fig. 1.—*Thaumantias globosa*, Forbes (*Phialidium variable*, Hæckel), with parasitic Halcampa; nat. size.

„ 2.—The same; magnified 4 diameters.

„ 3.—Parasitic larva of *Halcampa chrysanthellum*, older than that of fig. 1; magnified 5 diameters.

„ 4.—Oral disc of a still older larva, with eight tentacles, but twelve mesenteries, and showing the siphonoglyphe.

„ 5.—Oral aspect of larva with extended mesenteries; about 5 diameters.

„ 6.—Transverse section of larval Halcampa through the middle of the œsophagus (stomodæum).

„ 7.—Transverse section of larval Halcampa through the lower portion of the œsophagus (stomodæum).

„ 8.—Transverse section of larval Halcampa immediately below œsophagus (stomodæum).

Fig. **9.**—Transverse section of larval Halcampa in the gastric region.

,, **10.**—Transverse section of young larva of *Aptasia diaphana* (after R. and O. Hertwig).

,, **11.**—Transverse section of slightly older larva of *Aptasia diaphana* (after R. and O. Hertwig).

,, **12.**—Transverse section of adult *Edwardsia tuberculata* through the œsophagus (after R. and O. Hertwig).

,, **13.**—Transverse section of adult *Alcyonium digitatum* through the œsophagus.

,, **14.**—Transverse section of adult *Funiculina quadrangularis* through the œsophagus (after A. Milnes Marshall).

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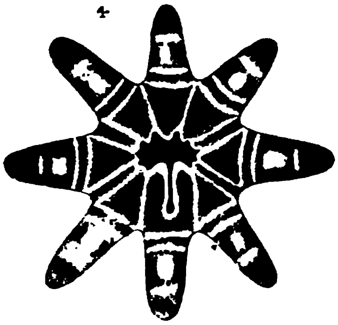

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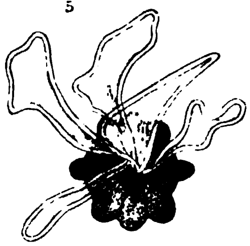

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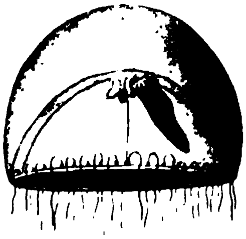

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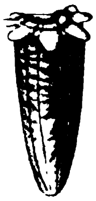

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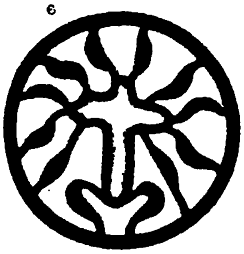

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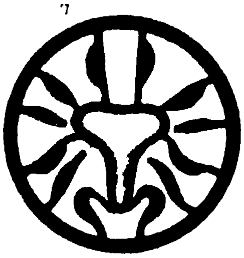

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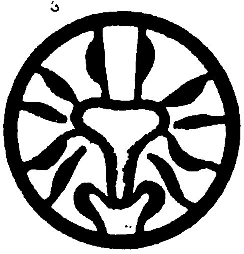

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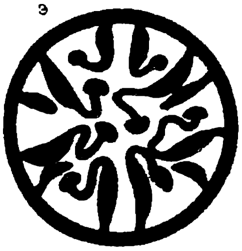

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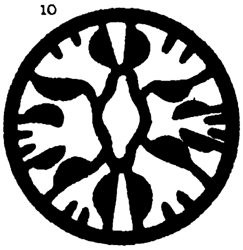

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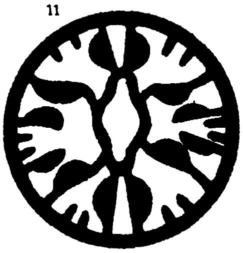

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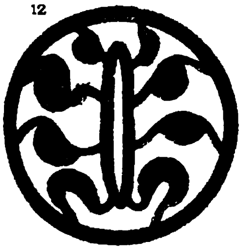

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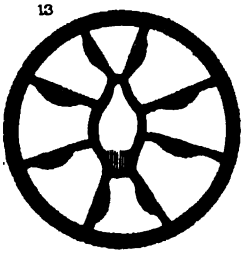

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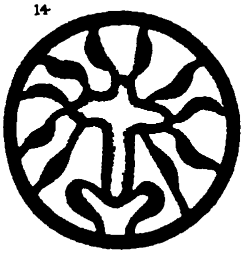

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SHEEP MYIASIS IN SOUTH-WEST SCOTLAND, WITH SPECIAL REFERENCE TO THE SPECIES INVOLVED

BY

A. J. HADDOW, B.Sc.

AND

R. C. MUIRHEAD THOMSON

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SHEEP MYIASIS IN SOUTH-WEST SCOTLAND, WITH SPECIAL REFERENCE TO THE SPECIES INVOLVED

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(With Three Figures in the Text)

Introduction

The increasing seriousness of sheep myiasis in Scotland, Australia and South Africa has, of recent years, claimed the attention of many workers. During the past 4 years the sudden and alarming aggravation of this pest in Scotland has attracted the notice not only of scientists and of the agricultural world, but of the lay public in general—a state of affairs clearly indicated by the extensive correspondence on this subject conducted by the *Glasgow Herald* during 1934–5. A significant feature has been the appearance of sheep maggot in areas hitherto untouched, while the position of sheep farming in the more seriously affected districts has become increasingly precarious. While, in most farms, the number of fatalities is still relatively small, extreme cases are noted from time to time, as, for example, that of an Argyllshire farm which lost 81 sheep out of 1000 in one season, despite the attentions of three shepherds. In the same year (1934) another farm in the same district lost 26 sheep out of 500. Of even more widespread importance is the cumulative damage to fleeces and to the sheep themselves, with resulting loss of condition and market value. The work of shepherds, moreover, is becoming excessively heavy, necessitating careful examination of the flock for maggot at least once daily during the fly season. Even such precautionary measures have, in some districts, proved entirely ineffective in preventing the occurrence of advanced cases, especially where the sheep are scattered over a large area of rough and bracken-covered country.

While in Australia and South Africa the volume of literature on sheep myiasis has been steadily accumulating, the number of independent investigations in Britain is still comparatively small. This is especially true of Scotland, the most seriously affected part of the British Isles. Since the classic survey by MacDougall in 1909, little of importance transpired until the beginning, in 1932, of the intensive researches of Prof. Ritchie and his colleagues at Aberdeen. It has been established by the above workers that *Lucilia sericata* Mg. is the almost exclusive cause of this condition in Scotland, some even asserting that it is definitely the only fly concerned. There are, however, occasional records of the presence of other species, notably *Calliphora erythrocephala* Mg. and *Lucilia caesar* (L.). It is one of the main objects of the present paper to emphasize the fact that the larvae of a few common carrion-flies do occasionally occur along with *L. sericata* as sheep maggots, at least in the area under consideration. While the part played by these is still of minor importance, there is every indication that they are following the general tendency of the Calliphorinae to forsake their former relatively harmless mode of life in favour of parasitism. In view of the implied danger, we have considered it important to attempt to estimate the incidence and role of these flies in certain districts, with the object of attracting the notice of authorities in a position to carry out a more widespread survey. As some of the results are not in agreement with the views of such a recognized authority as Prof. Ritchie, it may be noted that, throughout the entire work in 1933–5, the most stringent precautions have been employed to obviate any possibility of contamination of cultures.

While engaged in field observations on carrion-flies in 1933, one of the writers was impressed by their abundance in certain areas where sheep maggots were prevalent, and decided to find, by rearing experiments, whether they played any part in sheep myiasis. The results indicating that such was the case, it was decided to conduct a more extensive investigation, including parts of Ayrshire, Argyllshire and Arran. It was considered better to make a careful examination of the conditions obtaining on a small number of representative farms than to treat a large number more superficially. The farms included (Fig. 3) are: Craigends, Tayburn, Blackbyre, Low Rushaw, Muirside, Cairnhill and Drumtee, Cunninghame, Ayrshire; Glencloy, Glenkill and Machrie, Arran; Beachmeanach, High Crubasdale, and Barr Mains, West Kintyre; Carsaig and Arinafadbeg, Knapdale, and Fearnoch, Cowal. Material has been received from the following farms and districts outwith the area: Kirkton of Crawford, Lanarkshire; Auchmull and Gleneffock, Angus, and Strath, Flowerdale and Kerrysdale, Wester Ross. Fuller accounts of these farms are given below.

METHODS

While most previous work has been done on material sent by farmers for examination, it has been found more satisfactory to collect the larvae personally. This method, though necessitating more expenditure of time and labour, not only furnishes more complete information, but also ensures the purity of the sample, and that the larvae are taken from living sheep—a need not fully realized by many farmers. Practically every collection listed below was so made. In few cases were egg-masses collected, both on account of their fragility, and of the very unwieldy numbers of adults obtained by rearing even a few batches. In view of the high mortality frequently occurring in crowded cultures, it was found preferable, in the case of heavy infections, to rear representative numbers of each instar present than to take a large proportion of the entire number. The obvious drawback to this method is that larvae of a given species, present in small numbers in a heavy infection of *Lucilia sericata*, may be missed entirely, but where it is impossible to rear all the larvae present, the collection of a few of each stage gives a better chance of obtaining representatives of each original batch of eggs. The material was transferred directly from the sheep to the breeding jars, which were covered by four or five layers of close cotton, linen or muslin, secured by tight bands. The feeding materials employed were various. Minced beef was found to be rather unsatisfactory, owing to its property of forming a solid, comparatively dry cake. Fish, liver and brain, whole or mashed, or a mixture of the three, were found to be excellent. Equally good were the viscera of birds freshly shot for the purpose. As a substratum for pupation, fine sawdust, moss litter and bulb fibre were found effective. Earth and sand usually failed to give good results, as they were found to form dense masses round the puparia, with resultant high mortality. In every case it was found best to keep the material fairly moist—otherwise the resting larvae tended to dry up. In the many cases where larvae collected during late summer or autumn showed a tendency to hibernate, metamorphosis was accelerated by exposure to higher temperatures. It is both inconvenient and unsatisfactory to allow specimens to hibernate under artificial conditions, as no matter what precautions are taken, a large number usually die during the winter.

Though the apparatus devised by Keilin (1924) is beyond doubt the most satisfactory, as most of the work was done actually on the areas under investigation, and at considerable distance from any laboratory, it was necessary to use cages not only flyproof but portable. Various designs and materials were tried, the most efficient and generally used being the following: a large case, $26\frac{1}{2} \times 18\frac{1}{2} \times 13$ in. externally, was built of $\frac{3}{4}$-in. dove-tailed white mahogany, which does not warp readily, with a carrying strap passing over the sides and top (Fig. 1 A, B). The door, of nine-ply wood, formed one of the large sides, and was hinged below. The edge of the door was bevelled to fit closely when shut, and the fitting was further secured by a small, inwardly projecting rim within the door, ensuring an absolutely tight joint (Fig. 2). The whole was closed by means of three jam-hasps, which kept the door pressed tightly shut and flush with the surface. The case was adequately ventilated by means of nine slots $3\frac{1}{2} \times \frac{3}{4}$ in., six in the door, and three in the opposite side. These were covered by wire gauze of the finest grade, reinforced by a protective beading round the edge. Within were three sliding shelves of nine-ply wood, which could be entirely withdrawn, and, being provided with a deep flange at each end, could stand separately without disturbing the jars. Each shelf was perforated by seventeen staggered round holes. Glass tumblers, on account of their lightness and of their slight taper, which helped them to sit securely in these apertures, were used throughout as breeding jars. The tumblers and their covers were nowhere in contact with each other or with the walls of the case. This being a new type of apparatus, its flyproof qualities were tested thoroughly in the following way before it was used for rearing sheep maggots: twenty-one jars containing pure separate cultures of the larvae of six different species of carrion-fly were placed side by side in the box, along with a large number of open jars containing uncovered beef. The case was then closed, and left in a sheltered, sunny place, much frequented by blow-flies, for the space of 1 week. Large numbers of flies were attracted by the smell, but at the end of the week

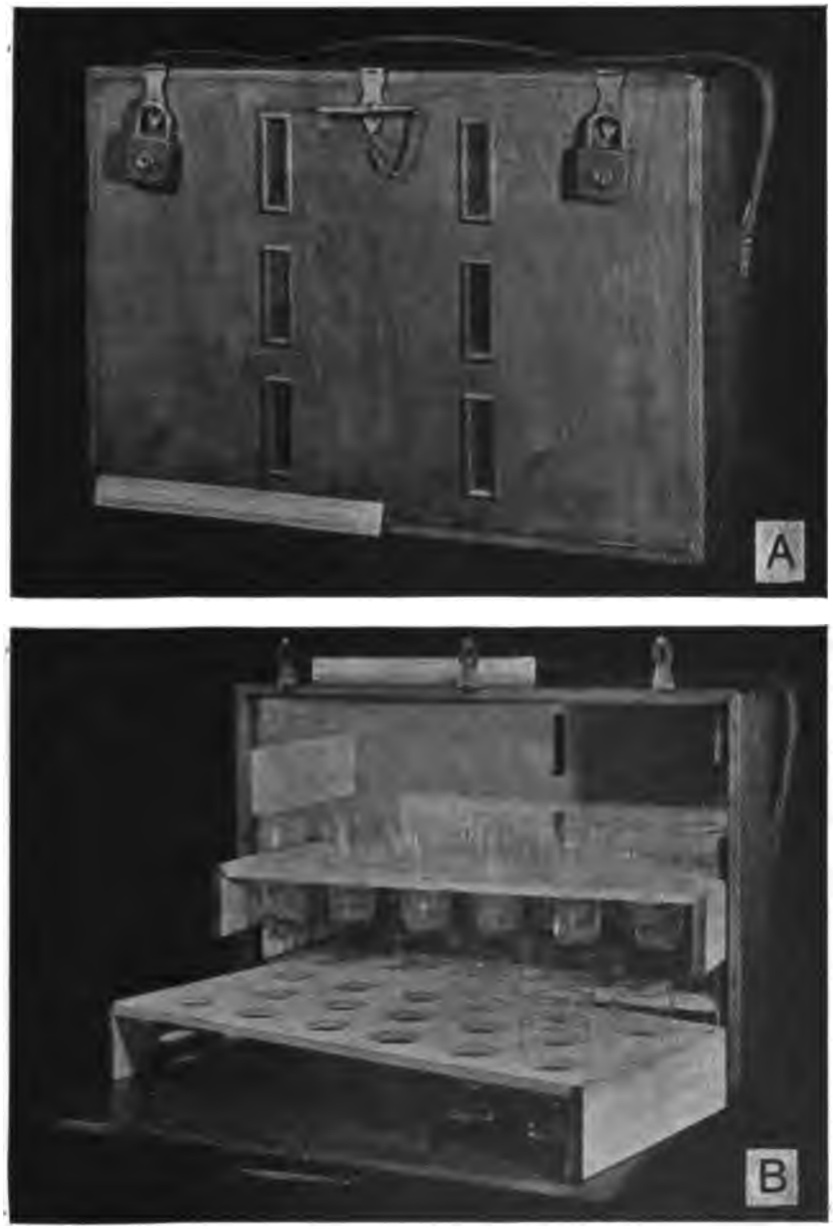

Fig. 1. Portable and flyproof breeding cage. A, closed; B, opened.

the case was found to contain no foreign eggs or larvae, the exposed beef being uncontaminated. All the cultures were reared out, and were found to have retained their purity. As an additional precaution during subsequent use, the box was kept indoors and in a dark place, and was examined within and without daily, as was each separate jar and cover. This case may be used for a variety of purposes by removing the sliding shelves, or by replacing them by plain shelves or pigeonholing made to fit. The entire weight of the apparatus when all the jars are full is 50–60 lb.

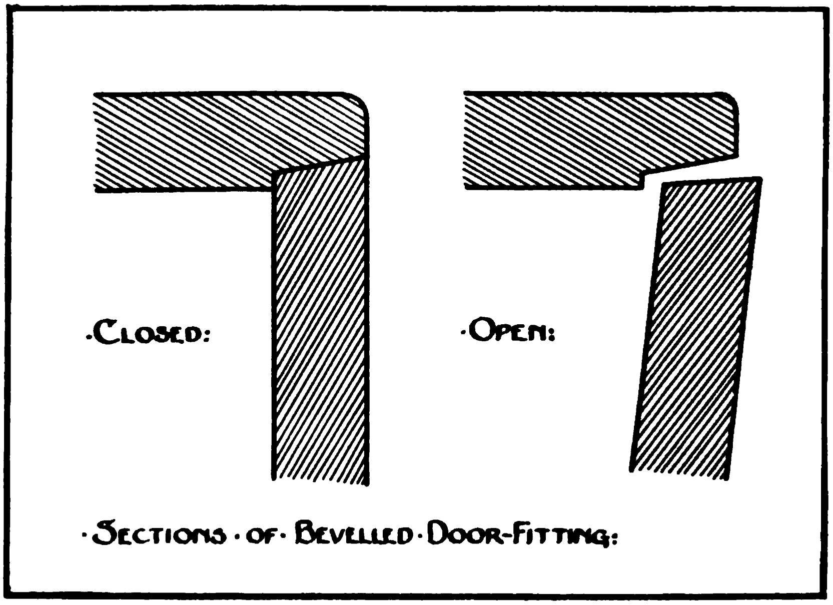

Fig. 2. Sections of door-fitting.

Preventive measures

Most of the farms concerned were of the small general type, none dealing exclusively with sheep. In few of them did the flock number more than 500. In four only were whole-time shepherds employed; in the remainder the sheep were supervised either by the farmer himself, or by a farm worker, in both the latter cases the general work of the farm occupying much of the day. In every case the sheep were examined at least once daily, by the individual concerned, during the fly season. The flock was invariably scattered over wide tracts of comparatively rough ground, rendering such supervision arduous and lengthy. Seldom could the shepherd be perfectly certain that no sheep had been overlooked—an important point when the amount of damage done by maggots in a single day is considered.

None of the preventive measures in use in the districts concerned can be considered adequate in dealing with the pest. As the present dipping regulations are concerned with the prevention of sheep-scab rather than of maggot, the first compulsory dipping of the year comes, in most districts, well after the beginning of the fly season. There is no doubt that in the majority of cases an earlier dipping would go far toward the prevention of these early cases which are so liable to progressive restrike throughout the summer. Such dipping as does take place is seldom efficiently carried out. We have observed a widespread tendency toward the use of over-strong solutions, with consequent skin irritation, loss of condition, and liability to strike. The time of immersion is usually much below the minimum prescribed by the maker of the dip. The majority of the troughs and pens are of the most primitive nature, and, a point of great importance, are rarely, if ever, cleaned. Two cases were noted where the dipping trough was never washed out, fresh dip merely being added to the existing fluid when the farmer considered this necessary. Few farmers realize the danger of overcrowding the sheep in small and dirty pens and drippers, with consequent fouling of the wool—a potent predisposing factor in strike. The conscientious use of recognized sulphur-arsenic dips, freshly made up at the correct strength, or even under strength, in clean troughs and with roomy dripping pens, have been found to be effective for a much longer time.

Where maggots are actually present on a sheep, many of the methods of removal are too drastic, causing inflammation and even the localized loss of wool. While many measures, such as the application of dilute dip, whale-oil and sulphur, Stockholm tar and the proprietary maggot-oils are satisfactory, we have often observed the unfortunate results produced by the use of concentrated carbolic and arsenical solutions or of crude paraffin. Among other substances used are dry earth, flowers of sulphur and sodium bicarbonate. Whale-oil and sulphur ointment, while forming a protective coagulum effective in preventing the irritation of open wounds by small muscids, anthomyids, etc., is of little use as a deterrent to maggot-flies. Owing to its property of cracking after drying and thus of damaging the underlying scab, it provides ready-made routes of entry to the already damaged tissues, nor does it seem to have a markedly harmful effect on the maggots themselves. Furthermore, healing is often retarded by the tendency of the sheep to bite at such dressings. While most farmers realize that maggots too rapidly killed *in situ* and not properly removed from the fleece will remain there rotting, and so attracting flies, most fail to appreciate the fact that living larvae rubbed out of the wool are, in many cases, able to complete their metamorphosis. Many shepherds, not wishing to kill the maggots in the wool, rub them out before applying the remedy, thus permitting a large number to escape. The advantages of clipping closely the wool round maggot wounds cannot be overemphasized; this process, besides rendering fresh egg-masses more obvious, seems to lessen the tendency to reinfection.

There is no question that indirect measures of control, when properly carried out, may be most effective, though this fact is seldom fully realized by those concerned. The process of "crutching", i.e. of shearing closely the breech and hindlegs down to the hocks, of clean and dirty sheep alike, as opposed to the usual method of clipping scouring sheep only, has been advocated (Robinson, 1935). We have seen this system employed with distinct advantage on two farms. The proper disposal of dead sheep and other carrion is of the highest importance as an indirect method of control. On very few farms is this effectively carried out. A common practice in the West Highlands is to allow the carcass to lie till the wool can be plucked off it—usually a period of some days. It is by this time, naturally, swarming with larvae, and the subsequent perfunctory burial, if any, in about 1 ft. of earth assists rather than hinders their development, as has been shown by Fuller (1932). Over the whole area the practice of allowing carcasses to lie exposed is prevalent—five such carcasses have been counted in the course of a mile's walk—most farmers firmly believing that the eventual burial destroys the maggots. In this connexion it is interesting to note a case recorded in the Press during the summer of 1935: some 200 sheep reported stolen from an area in Mid-Argyll were almost all eventually found by the police, not stolen but dead, mainly of myiasis—many of the carcasses being so infested as to be visibly moving, while a low, bubbling noise was audible from some little distance. On two farms only have we seen the treatment of carcasses with poisons before burial. Nowhere have we found any attempt to deal with small carrion, rabbits, birds, etc. A factor of importance and entirely outwith the farmer's control is the scandalous condition of many country slaughter-house "middens", where huge mounds of offal lie exposed and accumulating over long periods—ideal breeding grounds for large numbers of blow-flies. It is probable that such a state of affairs, favouring, as it must, the development of a carrion-fly population far too great for the available normal food supply, forces the insects concerned to seek new breeding places, such as living sheep. In this connexion it may be emphasized that many authorities, such as Froggatt (1904, 1915), attribute the first serious spread of sheep myiasis in Australia to a similar cause, namely, to the exposure of multitudes of carcasses as a result of the extensive campaign against the rabbit pest in that country. While in Britain the presence of large rabbit warrens will undoubtedly stimulate blow-fly increase owing to the number of bodies in their vicinity, we believe that their importance in aiding the over-wintering of flies has been exaggerated, definite evidence indicating that blow-flies pass the winter not as adults or pupae, but mainly as hibernating larvae. This point will be considered in greater detail below. Of recent years a few enterprising farmers have adopted the trapping system. We have noted two farms where the "Western Australian" trap as modified by de Vries (1933) is in use, and a few where bait is suspended over pails of sheep dip or lime, apparently with good effect.

SEASONAL INCIDENCE

While the fly season proper may be said to extend from the middle of June to the middle of September, various workers have recorded cases much earlier or later in the year. MacDougall (1909) noted cases in every month of the year except December, January and February, and Walton (1918) has found sheep attacked as early as March and April. In Ayrshire the pest seldom occurs before June. In Argyllshire we have records of sheep maggot in the first week of April, and have observed many cases in mild weather from the beginning of May onward. We have not noted the occurrence of myiasis in the districts concerned after the beginning of October.

The weather conditions influencing strike seem to be fairly constant—as noted by most workers, the flies attack most readily in warm, moist weather, while cold, wind and rain are unfavourable. On warm bright summer evenings, oviposition may occur at a comparatively late hour, on one occasion as late as 9 p.m. (British Summer Time) at the end of July. The mild winters and hot summers of 1932–3–4 have definitely contributed to the spread and increase of sheep myiasis in Scotland. As opposed to this, the cooler summer of 1935, though preceded by a winter as mild as the above, has shown, if anything, a decrease in the severity of maggot-fly attack in the districts studied.

TYPES OF COUNTRY

Little or nothing can be added to the existing accounts of the relation between sheep myiasis and the ground conditions involved. These have been fully discussed by Walton (1918) and others. We have found it a fairly general rule that the flies prefer low ground—sheep on high heathery country being less liable to attack. While avoiding the shade of woods, the flies seem to be most prevalent in sheltered spots, especially where much bracken is present. It is hard to overrate the importance of the latter as a predisposing factor in myiasis. Flies shelter in large numbers in bracken during heat—a situation much sought by the sheep also, for the sake of shade and coolness. In wet and cool weather, and by night, it acts as an efficient protection to many insects which would otherwise succumb. Equally important is the tendency of struck sheep to leave the rest of the flock and lie down in dense clumps of bracken, from which, in their peculiarly apathetic condition, they refuse to be dislodged by dogs. They are thus liable to be overlooked by the shepherd till they are past recovery, the carcasses of such victims remaining as hidden and unsuspected breeding grounds. It has been recognized by several authorities that wet sheep are particularly liable to attack, probably owing to the distinct odour of moist wool. Bracken remaining damp, as it does, long into the heat of the day, wets the neck and shoulders of sheep forcing their way through it, and thus renders these parts more attractive to blow-flies. On many farms the bracken, uncut from year to year, lies on the ground as a dense protective covering, in which a certain slight degree of warmth is probably generated by slow fermentation. Ground covered by such a layer is protected from extreme cold, and is less liable to freeze. This action is probably of importance in aiding the survival of hibernating prepupae during adverse winter conditions. On one Argyllshire farm we have noted a very substantial decrease in sheep maggot coincident with the cutting of large areas of bracken.

DAMAGE DONE TO SHEEP

On all the farms concerned the flock consisted of Blackface or cross-bred strains or both, sometimes a few Border Leicesters also being present. We have never obtained maggots from any of the last-named variety. On none of the farms studied were Cheviots stocked, but Cheviot owners state that this breed readily fall victims to blow-fly attack. In the west the Blackface is generally considered to be the most resistant variety.

With regard to the damage done to the sheep themselves, while it is known that any part may be affected, we have noted the high percentage of breech or tail strikes, mainly, but by no means always, on scouring sheep. This type of infection occurs mostly among ewes, lambs seeming more liable to be struck on the flanks and back. Rams seem to be most susceptible to attack on the neck and shoulders where the wool is thick. The horns of rams also seem to be peculiarly liable to infestation, especially if there has been any ulceration round the base, the face also being attacked in cases where there is friction between the horns and the cheeks during mastication. Wethers castrated by methods involving opening of the scrotum are frequently victims of "purse-strike" unless the wound has been carefully and completely sewed up. Maggots frequently occur where foot-rot is present, actually being introduced by some farmers with the object of clearing out the disease—a case of such treatment recently appeared in Court on a charge of cruelty. Beyond doubt this method is effective in clearing up the condition, but the larvae, unless removed in time, show a well-marked tendency to migrate up the leg and attack healthy tissue. As noted by Ratcliffe (1934) myiasis of the flanks is often induced by the soiling of the wool of this region by dirty or diseased trotters when the animal is lying down.

The majority of primary superficial lesions observed have been clean wounds, which, if not unduly disturbed, generally heal rapidly by granulation. If, however, as is often the case, the wound is much handled during treatment, there is definite danger of infection by pyogenic bacteria, especially by *Bacillus pyocyaneus* (*Pseudomonas aeruginosa* Migula), probably the commonest species in wool. The consequent formation of a discharging ulcer renders the wound extremely liable to restrike, with the added danger that the larvae, carrying the infection into the deeper tissues, may set up a fatal septicaemic or pyaemic condition.

STRIKE AND LIFE HISTORY

While absolute knowledge is as yet wanting as to the exact reasons for primary strike, several important predisposing causes have been recognized and, in addition to those already discussed, the following are important. It has been demonstrated by Holdaway (1932) that the main initial cause of body strike is bacterial activity in the wool. This, while obviously at a maximum where urine or faeces have soiled the fleece, also occurs in regions apparently clean and unaffected, and is the most probable explanation of strike in such parts. Where the wool is kept moist by water or sweat the extreme activity of the bacteria produces decomposition of the fibres or "wool-rot". This condition is known to be attractive to maggot-flies. It has been shown experimentally by Freney (1932) that artificially decomposed wool fibre (keratin) is lastingly attractive to the primary Australian blow-flies (including *Lucilia sericata*) to such an extent that it forms an excellent bait for traps. These facts show how warm, wet weather, hard driving or improperly trained dogs may, by wetting the wool, or causing the animals to sweat, stimulate bacterial growth and wool degeneration, and thus pave the way for maggot-fly attack.

Larvae in wool may either remain on the skin surface, causing comparatively little damage except to the fleece, or they may bore inward with more serious consequences. The method adopted seems to depend, in many cases, on the number of maggots present, and on the condition of the fleece. Larvae in small numbers seem to be able to obtain adequate nourishment from wool-yolk and from the superficial layers of the skin. This is seen mainly in the earlier parts of the year before the strikes are numerous. Such maggots differ in appearance from those which bore deeply, owing to the colour of the contents of the dorsal food reservoir—a fact which, together with their behaviour, has probably given rise to the widespread but erroneous belief that they belong to a different species, particularly active in late spring, *Calliphora* generally being the supposed agent. It follows from the above facts that a sheep with long, thick wool is more liable to exhibit this superficial type of myiasis, especially in the earlier months. Lambs and shorn sheep, on the other hand, though less liable to attack, usually suffer a much more extensive invasion of the tissues when struck. Larvae, when present in large numbers, almost invariably burrow more deeply, usually with serious consequences. Depending upon the part of the body struck, they may either penetrate at once, or may migrate some distance in the search for a soft or more easily invaded part, such as the flank or the fleshy part of the haunch. We have noticed that such migrating larvae usually tend to travel upward or forward, according to their original position. In hot weather, and under suitable conditions, hatching and larval growth may be greatly accelerated—in two cases the larvae had reached the third instar in a single day—one of these will be described below. Ratcliffe (1934) describes larvae which attained full growth in 48 hours at 90° F., the approximate temperature of sheep's wool near the skin. It has been stated by various workers that *Lucilia* larvae are not coprophagous. In several cases, however, they have been observed feeding on dung in wool, and, in one instance, a large batch were reared from the egg to maturity on such a diet alone, the larval period being much protracted and the adults small though perfect. In this connexion it is interesting to note that Bogdanow (1928) has reared eleven generations of *Calliphora erythrocephala*, normally a carrion feeder, on an exclusive diet of human faeces.

It has invariably been our experience that under natural conditions the winter is passed in a hibernating prepupal phase. On several occasions mature larvae of several species have been kept throughout the winter under a variety of natural and unnatural conditions, outside and indoors, pupation never taking place till April or May. In this particular we are entirely in agreement with Maldwyn Davies (1934). We consider the supposition of some authors that the winter is passed in the pupal state as most unlikely, nor have we seen adequate evidence adduced in its support. In no case have we found that the blow-flies can survive as pupae for more than 3 or 4 weeks. This fact is borne out by the following observations. It has been found possible to induce pupation in hibernating prepupae by one method only—continued exposure to high temperatures in hot houses. This is actually the method employed to hasten the emergence of autumn larvae, and so to reduce the high mortality prevalent among those permitted to hibernate normally. Pupation having been induced by such methods, it was found impossible to prolong its duration by any such means as re-exposure to normal winter temperatures, either inside or outdoors; under such conditions the adults either emerge after a pupal period of normal length or die within the puparium. The pupa, far from being a resistant instar as is usually supposed, has been found to be highly vulnerable, and susceptible to changes in temperature, humidity, etc., which are not injurious to the mature larva or prepupa, these stages being unaffected by relatively great variation in these factors. The prepupal phase can be prolonged almost indefinitely and is thus well adapted for hibernation.

We have never found parasites of any description in cultures of larvae taken from living sheep. This point has been noted by Maldwyn Davies (1934).

SPECIES OF FLY INVOLVED

It has been demonstrated by many workers that *Lucilia sericata* Mg. is almost exclusively the sheep maggot-fly concerned in Britain. Cases of attack by other flies have, however, occasionally been recorded. MacDougall (1909) mentions three cases of infection by *Calliphora erythrocephala* Mg. and three of the same species and *Lucilia sericata* mixed. In the same paper, reference is made to strike of sheep by *L. caesar* in Huntingdonshire and Kent, Theobald being the authority quoted. Hewitt (1914) mentions *L. caesar* as a sheep blow-fly. Walton (1918) states that *Calliphora erythrocephala* undoubtedly causes trouble, but gives no evidence. Maldwyn Davies (1934) notes two mixed infections of *Lucilia sericata* and *Calliphora erythrocephala* (the latter "in small numbers"). Ratcliffe (1934) states that *C. erythrocephala* is known to attack sheep. In the present investigation we have been concerned in an attempt to show that other species than *Lucilia sericata* do occur, in certain districts at least, as secondary agents of sheep myiasis following on or coincident with attack by this species, and that they may infest wounds and sores produced, in the first instance, either by disease or by larvae. The evidence indicates that these flies do not initiate myiasis unless some such conditions as foot-rot or a purulent sore be present. All the species involved are normally carrion-flies. We have found them almost exclusively in heavy or prolonged infections of *L. sericata*, in old wounds, etc. While the small numerical proportion of these flies, when present, and their rarity or total absence in myiasis in certain districts indicate clearly their occasional and secondary role, and render them, as yet, of minor importance, their presence leads to the belief that they may be following the world-wide parasitic trend of the Calliphorinae.

The flies concerned will now be considered briefly: *L. caesar* (L.) is a common carrion-fly over the entire area; we find it to be the most important of these secondary species and have recorded it in twenty-four cases of myiasis. *L. illustris* Mg., though not so common, is fairly well distributed in the west of Scotland. While in the past some workers have not recognized this species as distinct from *L. caesar*, the structure of the male genitalia as demonstrated by Séguy (1928) and Aubertin (1933) has placed the point beyond doubt. We have noted three cases of infection by this fly. *Calliphora erythrocephala* Mg., though one of the commonest species, and widely believed to cause sheep myiasis, we have found five times only. *C. vomitoria* L., though very common in some of the districts studied, has been reared from only two cases of maggot infection. *Protophormia terraenovae* R.-D. (=*groenlandica* Zett.) is a widely distributed British carrion-fly known by a variety of names such as *Phormia terraenovae*, *Protocalliphora groenlandica*, etc., the synonymy giving rise to much confusion. We have employed the above name (as given by Bezzi, 1922, and Séguy, 1928) after careful consideration of the evidence of morphology and priority. We have reared it in seven cases of sheep myiasis. In three instances a single larva of *Muscina pabulorum* Fallen, easily recognized by the characteristic posterior spiracles, has been taken from a living sheep and reared to maturity. In several of these cases we have found more than one of the species concerned occurring along with *L. sericata* on a single sheep. Fuller details are given below.

While cases of infection by the above-mentioned species of *Lucilia* and *Calliphora* are not altogether unexpected or unprecedented, especially in sores or wounds, the occurrence of *Protophormia* may occasion some surprise. In America, however, the closely allied species *Phormia regina* Mg. is well known as a sheep blow-fly in Texas (Bishopp, 1915), California (Herms, 1915), and British Columbia (Hearle, 1933). The presence of *Protophormia terraenovae* may be diagnosed by its characteristic habit of pupating in the fleece of the living sheep instead of in the ground. The puparia are readily identifiable by means of their well-developed lateral ridges and terminal processes—features sufficiently prominent to be recognized by a few observant farmers. We have seen these large and distinctive puparia on living sheep in various localities, but so far have reared the adults from larvae on sheep in the case of two farms only. The puparia become most evident on shearing, when they are sometimes found in fairly large numbers in the thick wool close to the skin.

The identification of species of *Lucilia* other than *L. sericata* has been confirmed in each case by microscopic examination of the genitalia. Miss D. Aubertin, of the British Museum, was kind enough to identify our first specimens of *L. illustris*.

COMPARISON OF AREAS WITH REARING RECORDS, ETC.

Within the area studied, a variety of conditions was encountered both in the type of country and in the methods of farming. The Ayrshire farms are all situated about 12 miles inland, at heights over 700 ft., round Fenwick Moor, a wide tract of rough grassland with little bracken. They lie on the borderline between rich arable and dairy-farming country and undulating moor. Farming in this district is of a high standard. The Arran farms, all close to the sea, are fairly low-lying. The ground is rough and is heavily covered with bracken and some heather. The remaining farms, in Argyllshire, like those in Arran, lie close to the sea, at varying heights. The country is very broken, and supports a dense growth of bracken and heather, and, in places, hazel scrub, etc. In Argyll farming is of a simpler nature than in Ayrshire.

The occurrence of the secondary flies was found to be related approximately to the above conditions. Comparatively rare in Ayrshire, their incidence increased in the western districts, reaching a maximum for the area in Kintyre and Knapdale. Their occurrence was obviously dependent on the type of country, efficiency of supervision, relative abundance of the species concerned, and local prevalence of myiasis which is much more severe in Argyll than in Ayrshire.

Below are given separate accounts of the various farms studied, with records of each case in which flies other than *L. sericata* were met. To save space no precise record is given of the many cases in which *L. sericata* alone was reared:

(1) *Sheep maggot in Ayrshire* (R. C. M. Thomson)

The farms to which particular attention was paid are all situated on the same type of moorland country, as described above. From the present point of view, the most important features of the ground are the open, undulating nature of the rough grassland, the absence of deep, sheltered glens, and the relative scarcity of bracken. In Ayrshire, as in many other parts of Scotland, there is a widespread belief that there are two distinct kinds of maggot or "mawk" associated with two different flies. In the early summer there is a "soft" "dung" or "black-headed mawk" supposed to be that of *Calliphora*, which is replaced later in summer by the more harmful "red-headed mawk" associated with *L. sericata*. Breeding records show that this hard and fast distinction is quite unfounded. *Calliphora* has not been recorded as a sheep maggot-fly in this area, and "black-headed mawks" of various different types have all proved to be those of *Lucilia sericata*. *Protophormia* has only been taken from sheep on one occasion, but all farmers and shepherds have, at some time or other, seen the black puparia in the wool of living sheep. Although widespread, it is evidently only of occasional occurrence on sheep. The secondary sheep maggot-flies play a less important part in Ayrshire than in any of the other areas investigated. Breeding records have shown that the vast majority of strikes are due to *Lucilia sericata*, and numerous cases of attack by this species alone have been obtained from the following farms: Craigends, Tayburn, Low Rushaw, Muirside, Blackbyre, and Cairnhill. Secondary sheep maggot-flies have been recorded in the following few cases:

Drumtee Farm. This is situated on a bleak tract of rough grassland, merging with moorland, near the north boundary of Aryshire. Sheep maggot is less severe here than in other farms of the area.

5. vii. 33. Several pupae and full-grown larvae of *Protophormia* as well as a single larva of *Muscina pabulorum* were taken from the wool of a sheep. This was evidently a secondary attack in which the primary larvae of *Lucilia sericata* had left the sheep in order to pupate, and progressive restrike had been checked by unfavourable weather.

Craigends Farm lies in a hollow in the moor, and the ground, though rough, is less exposed than at Drumtee.

10. vi. 33. Larvae of different sizes were taken from a mild case on the soiled hindquarters of a Blackface lamb. This was an unusually early strike for this farm. The flies recorded were:

L. sericata	...	22
L. illustris	...	7

Tayburn Farm is situated near Craigends, and has very similar ground and exposure. None of the several cases examined here during the summer was serious, and with a single exception they were found to be caused by *L. sericata* alone.

16. vii. 35. Several larvae were taken from a small patch on the hip of a Blackface lamb. The flies bred were:

L. sericata	...	23
M. pabulorum	...	1

Muirside Farm is situated on very exposed, undulating moorland and the sheep are over a much wider area than in the other farms. Maggot-fly was very troublesome here during the summers of 1933–4–5, but, owing to strict supervision, was kept well under control. At one time in July 1935 only four sheep out of 500 were struck. Several cases of attack by *Lucilia sericata* alone were recorded during the summer, and in only one instance did secondary flies appear.

29. vii. 35. The flies recorded were:

L. sericata	...	42
L. illustris	...	4

(2) *Sheep maggot in Arran* (R. C. M. Thomson)

In the summer of 1935 an investigation was carried out in three localities in Arran—Brodick and Lamlash on the east coast, and Machrie on the west coast. Sheep farming on a large scale is confined to these three districts, and the conditions obtaining there may be taken as fairly representative of the whole island. Here, as in most other places in Scotland, sheep maggot has become increasingly serious in the last few years, and has spread to the south of the island, where, till recent times, it was unknown. The farmers and shepherds of six farms recognize three different "mawks" as compared with the two of Ayrshire, and associate them with three distinct flies, *Calliphora*, *Protophormia*, and *Lucilia*. Only the two latter species, however, have been recorded from these farms, and *Calliphora*, if it does occur at all, must be a very rare sheep maggot-fly in this area. All those concerned with the actual handling of sheep have seen the black puparia of *Protophormia* in the wool of living sheep, and, although it has not occurred in any of the breeding records for this district, it is evidently a fairly generally distributed, if never abundant, sheep maggot-fly. Larvae were obtained from three farms, and the separate records are considered in more detail below:

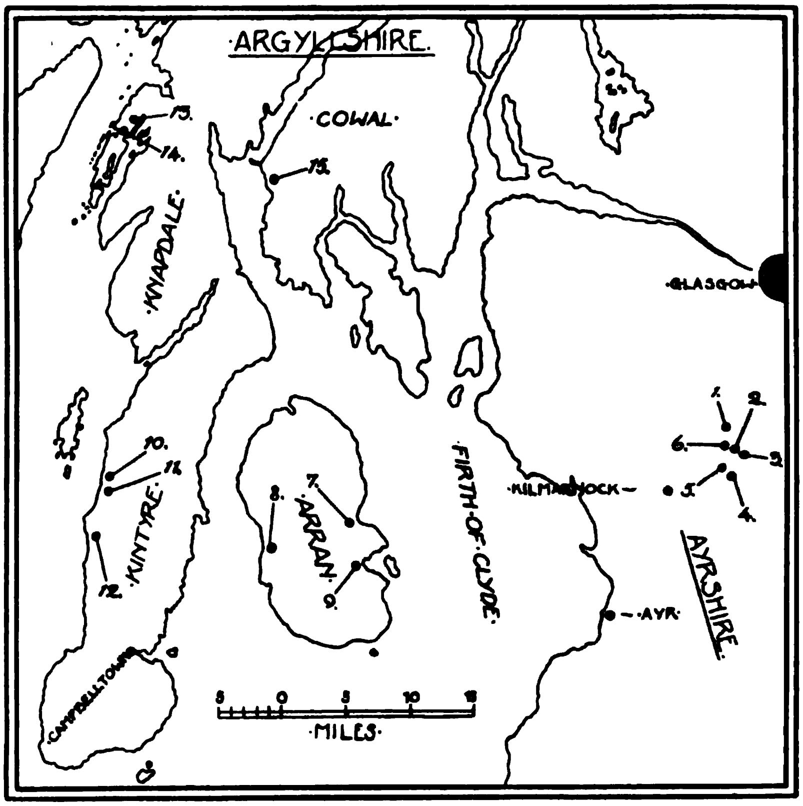

Fig. 3. The collecting area. Names of farms: 1, Drumtee; 2, Tayburn; 3, Craigends; 4, Muirside; 5, Low Rushaw; 6, Blackbyre; 7, Glencloy; 8, Machrie; 9, Glenkill; 10, Beachmeanach; 11, High Crubasdale; 12, Barr Mains; 13, Arinafadbeg; 14, Carsaig; 15, Fearnoch.

Glencloy Farm. This farm is situated in a long, narrow glen, running down to the sea, and is protected on three sides by steep hills. Owing to the sheltered position, the glen is very hot in summer, and this, together with the rough nature of the ground and the general covering of dense bracken, make it particularly difficult for the shepherd to keep the maggot-fly under control.

9. viii. 35. Larvae of various sizes were taken from an extensive, open wound along the

back of a Blackface ewe which had previously been attacked and subsequently cleaned of maggots. The flies recorded were:

L. sericata ...	31
L. caesar ...	47

Glenkill Farm. This farm is situated near the sea at the beginning of a wide glen. The ground, though rough and bracken-covered, is much less sheltered than at Glencloy.

8. viii. 35. Larvae of different sizes were taken from a mild case on the tail region of a Blackface ewe. The flies bred from these were:

L. sericata ...	59
M. pabulorum ...	1

Machrie Farm lies at the edge of a wide stretch of flat country on the west coast of the island. The ground and exposure are very like those of the west of Kintyre, and the slopes of the hills are similarly heavily covered with bracken. Sheep maggot was not so severe as in Glencloy, but continued much later in the season, in spite of cold, wet weather. Three samples of larvae were obtained, all of them yielding small proportions of *Lucilia caesar*.

9. viii. 35. The flies recorded were:

L. sericata ...	116
L. caesar ...	1

11. ix. 35. The flies recorded were:

L. sericata ...	68
L. caesar ...	8

17. ix. 35. The flies recorded were:

L. sericata ...	41
L. caesar ...	6

(3) *Sheep maggot in Argyllshire* (A. J. Haddow)

In the districts studied, the main enemy of the farmer is probably the dense growth of bracken, which, together with the moist, mild climate, favours the increase of sheep blow-flies. Myiasis of sheep in this area is probably as serious as anywhere in Scotland, fatal cases being not infrequent, and heavy infestations an everyday occurrence. The shepherd's task is rendered difficult by the fact that he is usually expected to perform other duties besides care of the flock. The country being rough, the difficulties of supervision are great. At Beachmeanach, *Protophormia* is abundant, and from this farm only has it been recorded as a maggot-fly. The puparia have however been observed in wool at Cleit, West Kintyre. On Carsaig Farm, Knapdale, an effective campaign against sheep maggot during 1935 resulted in a reduction of fatalities by over 90 per cent as compared with 1934. The total number of strikes was also very substantially reduced. The measures employed were: cutting of bracken, building of large pens, use of an isolation field for bad cases, regular use of clean and properly mixed dip, crutching, prompt carcass burial, daily examination of the flock by an efficient shepherd, and immediate and careful treatment of all struck sheep. Carcass burial elsewhere was usually rather tardily and inefficiently done. Many Argyllshire farmers firmly believe that young larvae of *Lucilia sericata* belong to different species from the fully grown ones. Below is given a record of cases in which flies other than *L. sericata* were reared:

Beachmeanach Farm, West Kintyre, consists of a stretch of flat shore fields with dense tracts of bracken and couch grass, an intermediate zone of hill pasture with much bracken and whin, and a considerable area of heathery hillside. The sheep, which are kept mainly on the high ground, gather in hot weather in a narrow, bracken-choked glen. In spite of careful dipping and supervision, maggots cause much trouble on this farm. Blow-flies of many species are peculiarly abundant here.

3–11. vii. 34. Larvae from various cases were reared in rather limited quarters, with consequent high mortality. The following emerged:

L. sericata	3
Pr. terraenovae ...	16

30. vi. 35. Larvae in large numbers and all stages were taken from a large wound on the haunch of a ewe. The fleece was much soiled. There was a considerable bare area on the flank. The following emerged:

L. sericata	55
L. caesar	2
Pr. terraenovae ...	1

5. vii. 35. Considerable numbers of maggots in all stages were found in a wound 3 in. square on the haunch of a ewe. The wound, which was necrotic, penetrated almost to the bone. The flank and back were also infested. This case terminated fatally. The following emerged:

L. sericata	23
Pr. terraenovae ...	13

7. vii. 35. Larvae were collected from a heavily infested ewe. They were present in vast numbers all over the back and the left haunch and flank. There was most extensive destruction of the superficial tissues. The fleece in these regions was hanging in loose strands. The following emerged:

L. sericata	1178
L. caesar	54
Pr. terraenovae ...	9

8. vii. 35. An old ewe was found to have a maggot wound extending from the hock upward to the middle of the back. The larvae were mainly in the first stage. The following emerged:

L. sericata	225
L. caesar	12
Pr. terraenovae ...	1

10. vii. 35. Larvae in moderate numbers and all stages were taken from the haunch of a hogg. The skin was bare but unbroken. The wool was much soiled with urine and faeces. The following emerged:

L. sericata	248
L. caesar	7
Pr. terraenovae ...	18

10. vii. 35. Both haunches of a ewe were found to be covered with larvae, mainly in the first stage. The skin was bare but unbroken. The wool was matted with urine and faeces. The following emerged:

L. sericata	246
L. caesar	14

27. vii. 35. An old maggot wound in the flank of a ram was found to be full of eggs and first-stage larvae. Clean wounds were also present on the other flank and both sides of the neck. The wounds were disinfested, clipped and treated with dip. The next day the same wound and a new one below it were surrounded by eggs and swarming with larvae *already*

in the third stage (i.e. within 1 day). The following day the wounds were infested, and a new one was present between the shoulders. The following emerged:

L. sericata	171
L. caesar	4

High Crubasdale Farm, West Kintyre, is situated at a height of 300–400 ft. on an exposed, heathery hillside. Sheep blow-flies are not a serious problem here. The stretches of pasture are fairly clear of bracken.

6. vii. 35. Moderate numbers of third-instar larvae were collected from the tail and haunches of a scouring ewe. The skin was unbroken. The following emerged:

L. sericata	7
L. caesar	14

6. vii. 35. A few third-stage maggots were found on the tail of a scouring ewe. The skin was unbroken. The following emerged:

L. sericata	13
L. caesar	1

Barr Mains Farm, West Kintyre, closely resembles Beachmeanach (see above). It is, however, much more exposed, and the hill-pasture zone is rather marshy, with extensive reed-beds. The farm is divided by a deep glen, containing much bracken.

22. ix. 34. Two very small egg clusters were taken from the margin of an old, healed maggot wound on the haunch of a clean lamb. The following emerged:

L. caesar	22
C. erythrocephala	4

23. ix. 34. Four small groups of larvae were taken from wounds between the hindlegs of a scouring ewe. In consequence of previous attacks, most of the wool of the back was missing. The larvae were all in the first and second instars. The following emerged:

L. sericata	58
L. illustris	28

23. ix. 34. Three clusters of eggs were removed from the haunch of a clean lamb. The following emerged:

C. erythrocephala	500 approximately.

Carsaig Farm, North Knapdale, occupies a narrow glen. The floor of the glen is sheltered and marshy; the walls are covered with abundant bracken and heather. Pasture is rather scanty. There are very few trees.

4. viii. 35. Maggots in large numbers were taken from a wound of 3 in. diameter on the haunch of a clean wether lamb. The skin was rendered spongy by multitudes of small holes. The surrounding fleece was also heavily infested. The following emerged:

L. sericata	23
L. caesar	84

27. viii. 35. Moderate numbers of larvae in all stages were taken from the trotter of a ewe suffering from foot-rot. There had been extensive penetration of the diseased part. The following emerged:

L. sericata	1
L. caesar	161
C. erythrocephala	2

A few clusters of eggs were collected from the flank of the same ewe, at a point which would be in contact with the affected trotter when the animal was lying down. The following emerged:

L. caesar	202
C. erythrocephala	1
C. vomitoria	1

Arinafadbeg Farm, North Knapdale, occupies a steep hillside. The higher parts are heathery. Below is a belt of hill pasture, with dense tracts of bracken, hazel scrub and pines. The low ground consists of sheltered fields surrounded by woods of hazel and pine. The whole is fairly sheltered. Sheep maggot is very troublesome on this farm.

29. v. 35. A large number of larvae in all stages were found feeding close to the skin on the haunches of a scouring ewe. The following emerged:

L. sericata	441
L. caesar	21

30. v. 35. A large number of larvae were removed from the hindquarters of a ewe. The fleece was wet with urine. The tail was much damaged. The following emerged:

L. sericata	159
L. caesar	7

12. viii. 35. The base of a ewe's horn was found to be full of maggots. They had penetrated within and around the horn, which was rather loose. The wound was discharging. The following emerged:

L. caesar	51

12. viii. 35. A large area above the tail of a scouring ewe was found to be infested with small larvae, which were just penetrating the skin. The following emerged:

L. sericata	4
L. caesar	71

27. viii. 35. Vast numbers of larvae were taken from a weakly wether lamb. The head, neck, shoulders, hindquarters and belly were swarming with larvae, many thousands being present. The case terminated fatally the next day. There was a heavy mortality in the cultures. The following emerged:

L. sericata	10
L. caesar	143
C. vomitoria	22

28. viii. 35. The cheek and ear of a wether lamb were found to have been almost totally destroyed by maggots. There had been extensive invasion of the deep tissues. A foul discharge was present. The lamb died a few hours later. The following emerged:

L. sericata	13
L. caesar	97

Fearnoch Farm, Kilfinan, Cowal, consists of heathery hill land, with dense belts of bracken, a few flat fields, and some woods. During 1933–4 sheep myiasis was prevalent, but few cases occurred in 1935.

17. viii. 35. A moderate number of larvae were taken from above the tail of a scouring ewe. The skin was just breaking. Much blow was present. The following emerged:

L. sericata	39
L. caesar	14

(4) *Farms outwith the area* (A. J. Haddow)

Material from Wester Ross and from Gleneffock, Angus, consisted of *L. sericata* alone.

Kirkton of Crawford, Lanarkshire.

25. ix. 34. A batch of larvae, taken from a single sheep was sent by hand. Approximately 200 *Calliphora erythrocephala* emerged.

Auchmull Farm, Glenesk, Angus, is situated in a fairly wide, grassy glen, with much bracken and some trees.

4. vii. 35. A consignment of larvae was received by post. The following emerged:

L. sericata	1026
L. caesar	9

SUMMARY

1. Sheep myiasis was investigated in certain districts in Ayrshire, Arran and Argyllshire.

2. Larvae from cases of myiasis were collected and bred out in flyproof apparatus.

3. The fly season was found to last approximately from May till September, reaching its height in July and August.

4. The western districts were found to be the most heavily affected.

5. The flies were found to be partial to low-lying, sheltered ground, especially where much bracken was present.

6. The methods of carcass burial were found to be primitive and inadequate. In most of the farms dipping was carried out without sufficient attention to cleanliness and adequate time of immersion.

7. The hibernation of various species of blow-fly was studied. They were found to pass the winter as prepupae, pupation never occurring normally till April or May.

8. Several species of carrion-feeding blow-fly besides *Lucilia sericata* were found to be causing secondary myiasis, occasionally and in small numbers, in certain districts, mainly in the west. The species involved were: *Lucilia caesar*, *L. illustris*, *Protophormia terraenovae*, *Calliphora erythrocephala*, *C. vomitoria* and *Muscina pabulorum*. Four of these species *Lucilia illustris*, *Protophormia terraenovae*, *Calliphora vomitoria* and *Muscina pabulorum* have not previously been recorded from sheep. Records of cases in which these flies occurred are given.

We wish to thank Dr R. A. Staig of the Department of Zoology, University of Glasgow, for valuable help and advice, Mr W. H. R. Lumsden for much practical assistance and for material from Angus and Wester Ross, and, above all, the farmers and shepherds whose friendliness and co-operation have enabled the work to be carried out.

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Observations on *Anopheles marteri*, S. & P.

Estratto dalla "*Rivista di Malariologia*,,
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PROPRIETÀ LETTERARIA

Observations on *Anopheles marteri*, S. & P.(1)

(Diptera, Culicidae)

J. Hadjinicolaou

In July 1936, the writer found larvae of *A. marteri*, S. and P. in a permanent stream on Mt. Itis in central Greece, at an altitude of about 300 meters.

This species was first described by SENEVET and PRUNNELLE (5,6) who found it breeding in company with *A. claviger* (*bifurcatus*) in the Tell mountain region of Algeria in April 1927. They gave a description of the larva, pupa, and male genitalia. The egg stage was not observed. LANGERON and GALLIARD (2) reported in March 1933 that when revising a collection of mosquito larvae made in 1925, two types new to Corsica were found, one being a larva of *A. marteri*. SHANNON (7) found *A. marteri* on Mt. Pangeon, Macedonia, at an altitude of about 1000 meters in October 1932 and again in the following month of May. He also found this species in southern Bulgaria, in the region of Mt. Beles at an altitude of about 500 meters, not far from the Greek border, in July 1935 (unpublished record).

A. marteri is a species wich breeds in the cold, clear water of mountain streams. It prefers running water and is found usually near waterfalls and under overhanging rocks. The larvae are seldom found in standing water and in such cases the small residual pools are near a running stream wich has decreased in volume. The species has a definite preference for deeply shaded breeding places and is rarely found in water exposed to the sun.

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The writer is greatly indebted to Dr. M. C. BALFOUR, under whose direction this work was done, for reading over the manuscript and for many criticisms and suggestions; and to Dr. G. GEORGOPOULOS of the School of Hygiene of Athens who helped in carrying out some of the experiments.

Breeding places of *A. marteri*, Mt. Itis, Central Greece.

LARVAL STAGES.

In order to obtain information about the life cycle of *A. marteri* several hundred larvae were collected in July. The first attempt to raise them in the laboratory was unsuccessful. Larvae of all stages kept in bowls (30 larvae in each of four enameled bowls, 20 cm. in diameter and 15 cm. depth), were supplied with a nutritive mixture of dried blood serum and flour but all of them died before reaching the pupal stage. The room temperature varied from 26.-33° C and the water temperature was often above 25° C. The temperature of the water in the natural breeding place was relatively constant, between 17 and 19° C. Presumably the temperature factor accounted for the failure.

A second effort to raise larvae was made in a cooler, basement room, wich was semi-underground and where the room temperature was fairly constant at 26° C. Larvae were put in galvanized iron pans (66 × 35 × 20 cm.) and the water was kept cold by the use of an ice bath. The water temperature, recorded three times daily, ranged from 19° to 24° C. It was also arranged that water drip from a faucet into the larval pans. Small rocks and plane tree leaves, as well as the nutritive mixture, were introduced to simulate a natural environment. Under these conditions, the larvae developed normally, mortality was low, and hundreds of pupae and adults were bred out.

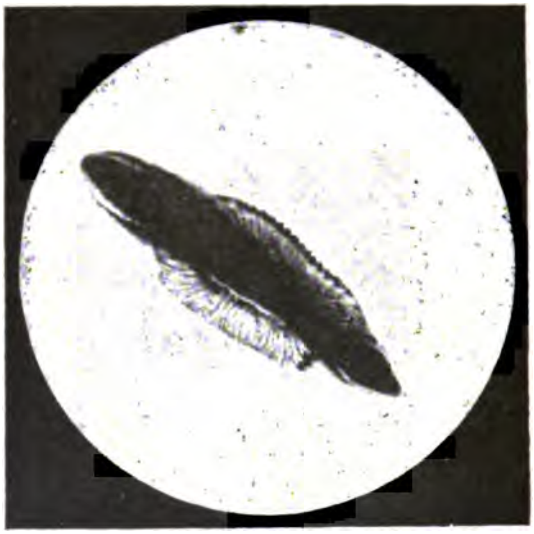
Another laboratory experiment was made with 570 larvae of the II, III and IV stages to determine the duration of the larval and pupal stages. Because of difficulties in classifying the different stages and also in finding all larval skins, even in batches of 20 larvae, these observations did not lead to conclusive results. However, when the eggs of *A. marteri* were found later in nature and their identity proved, this question was again examined. From a number of eggs collected on Aug. 8th, seven eggs hatched on the following day and two larvae were carried through to adults of *A. marteri* under the above laboratory conditions. The larval stages were established by finding the larval skins ; the I, II, III, and IV stages required 3, 5 1/2, 3 1/2 and 6 days, respectively. The pupal stage lasted 2 days and the development from egg to adult was thus completed in 20 days under the given conditions.

In another trial, 118 fourth stage larvae, which pupated, passed through the pupal stage on an average of slightly less than three days, the period varying from one to four days. Ninety-four adults emerged.

Senevet (4) reported from Algeria in 1932 that he found the first larvae of *A. marteri* on April 30th and the last ones on June 23rd. In Greece, we found larvae for the first time in the breeding place on Mt. Itis on June 22nd and continued to find them there in abundance until the early part of October. During the summer months the larvae were so plentiful that one could frequently collect more than a hundred of different stages in one dip. On October 27th, the number of larvae had materially decreased and in early November they disappeared almost completely. A long search resulted in finding only five or six large larvae. During late October and early November heavy rains occurred which probably washed most of the larvae away. While eggs were constantly found during August, September and early October, none were found in late October or early November.

EGGS OF « A. MARTERI ».

On August 1st, a number of eggs were found in the breeding place of *A. marteri* on Mt. Itis. These eggs, obtained by the use of a mitten (1) and also observed floating on the water surface in the larval dipper, were brought to the laboratory. Using the above technique of rearing, they hatched and were bred through to the adult stage. The full-grown larvae and adults had characters which agreed with those given by SENEVET and PRUNNELLE. The special character of two opposite spines on the tip of the penis, mentioned by MARTINI (3) for *A. marteri* in his key for the determination of species of the subgenus, *Anopheles*, was also noted.



Egg of *A. marteri.*

The eggs of *A. marteri* are uniformly black and resemble in some degree those of *A. claviger* (*bifurcatus*). They may be distinguished by the very large floats, which are more than three-fourths of the total egg length ; the width of the float at its broadest central part is one and one-half times the width of the ventral surface of the egg which is seen between the two floats. The number of ribs, counted on the floats of 10 eggs, averaged 26, varying from 23 to 29 ribs per float. The intercostal membrane of the float structure of *A. marteri* is moderately ridged or rough while that of *A. claviger* (*bifurcatus*) is smooth.

FEEDING HABITS AND HIBERNATION OF ADULTS.

The feeding habits of the adults of *A. marteri* are not known. It seems likely that this species remains in the wooded regions and seldom enters stables or bedroms for food or shelter. In the middle of August, when larval breeding was at its maximum, we searched in the stables and bedrooms of a village not more than 500 meters distant from the breeding place, and no adults were found. A number of adult males and females were observed on the same day resting in tree and rock cavities near the stream and some adults were caught in flight.

On Sept. 26th, an empty female was caught in a stable in the village of Zeli by our field assistant, M. RENDOUMIS. Later an adult female (probably *A. marteri* but not confirmed), which contained partly digested blood, was caught in a stable at Platystomo. In November, a colleague, Dr. SPHANGOS, captured 23 female and 9 male *marteri* on the walls of an underground reservoir during a visit to Aedipsos, on the island of Euboea. On a subsequent visit to this village in February, 5 females were caught in a stable and one female was found in a bedroom. Some of these specimens were empty while others contained fat bodies.

With a view to testing the feeding habits of *A. marteri* about 30 females were liberated in August in a screened room where two persons were sleeping. These mosquitoes were recaptured on the following day ; all were empty and showed no evidence of having fed. A repetition of this test on several occasions gave similar results.

Fifty adult specimens, males and females, were placed in a screened cage (75 × 45 × 40 cm.) and offered repeatedly a human arm as bait. No biting occurred. After several attempts with different batches of female *marteri*, which were put in round cages, (12 cm. diameter and 2 cm. wide), 2 of the 12 adult *marteri* took human blood when placed in contact with the exposed abdomen.

SUMMARY AND CONCLUSIONS.

These observations provide additional information about *A. marteri* and its biology.

1) Larvae of *A. marteri*, S. and P. were found in abundance from July until early October in a mountain stream of Mt. Itis, in Central Greece, at an altitude of about 300 meters.

2) Eggs of *A. marteri* were found in nature and are described for the first time.

3) In Algeria, the larvae of this species, according to SENEVET's observations, appear early in the spring and disappear completely about the end of June. In Greece, the larvae have been found until early November.

4) The life cycle from egg to adult under laboratory conditions in August with an average room temperature of 26° C. and water temperatures ranging from 19. to 24° C., was completed in 20 days.

5) Limited observations regarding hibernation provide some evidence that this species passes the winter in the adult stage; the larvae disappear in November, the time depending on the onset of the winter rains.

6) Since *A. marteri* has not been found in shelters, except during the period of hibernation, and because it feeds rarely on human bait, it seems unlikely that this species feeds on man.

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RIASSUNTO.

L'A. reca un contributo alla conoscenza di *Anopheles marteri*, S. & P. I rilievi furono eseguiti nella Grecia centrale. Si trovarono larve abbondanti da luglio al principio di ottobre, in un corso d'acqua del Monte Isis, ad altitudine di circa 300 m. Le larve furono presenti fino al principio di novembre. (Secondo osservazioni di SEVENET, in Algeria, le larve di *marteri* compaiono al principio della primavera e scompaiono del tutto verso la fine di giugno). Furono repertate le uova in natura e vengono descritte per la prima volta. Il ciclo vitale dall'uovo all'imagine fu completato in 20 giorni durante l'agosto, in condizioni di laboratorio, cioè a temperatura dell'aria di 26° C. in media ed a temperatura dell'acqua tra 19° e 24° C. Le larve scompaiono in novembre, in rapporto con le piogge invernali. Osservazioni limitate tendono a provare che la specie attraversa l'inverno allo stato adulto. Siccome *A. marteri* non è stato trovato entro ripari che durante il periodo d'ibernazione, e siccome di rado aggredisce l'uomo, sembra improbabile che esso sia vettore di malaria.

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[illegible], Ph.,
Die Lebensweise und Bekämpfung
der Buchsgallmücke.
15

Separatabdruck aus dem «Schweizer Garten» No. 5 vom 20. Mai 1936

Hommage de l'au

Die Lebensweise und Bekämpfung der Buchsgallmücke.

(Monarthropalpus buxi Lab.)

Seit einigen Jahren werden bei uns die Buchspflanzen immer mehr von einem Schädling befallen, der das Blattwerk zerstört, die Pflanzen unansehnlich macht und häufig zugrund richtet. Es handelt sich um die *Buchsgallmücke,* ein kleines, zartes Insekt aus der Familie der Cecidomyidae. Dieser gefährliche Buchsschädling wurde 1912 sehr eingehend von Chaine[1]) beschrieben; er ist besonders in Frankreich und in Amerika sehr stark aufgetreten, aber auch in andern Ländern und in der Schweiz bekannt. Zufolge der kurzen Flugzeit von 3 bis 4 Wochen, sowie der geringen Körpergrösse von 3—4 mm, werden die Mücken kaum beachtet. Während 11 Monaten des Jahres ist der Schädling in geschlossenen Blattminen versteckt. Die genaue Kenntnis der Lebensweise ist für eine wirksame Bekämpfung ausserordentlich wichtig.

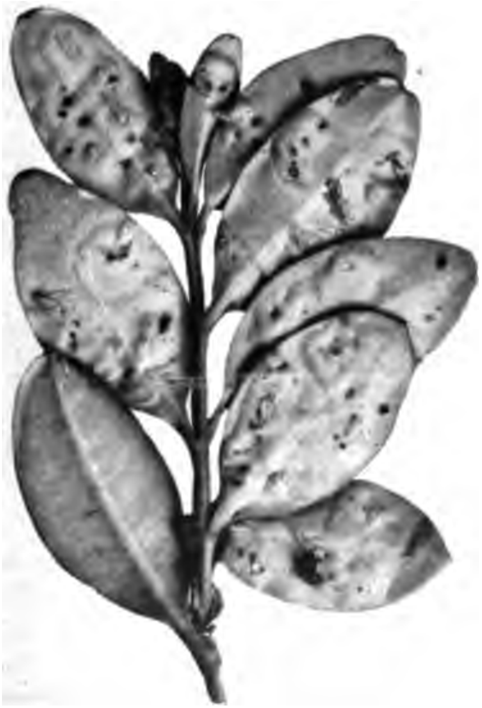

Fig. 1: Buchszweig, sehr stark durch Buchsgallmücken befallen.

Der Beginn der Flugzeit und somit der Eiablage wird durch die klimatischen Ver-

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[1]) J. Chaine, La Cécidomyie du buis (Monarthropalpus buxi Lab.), Morphologie, Biologie, Dégâts, Traitement. Ann. des Sc. nat. zool., 9e série 1912 XVII.

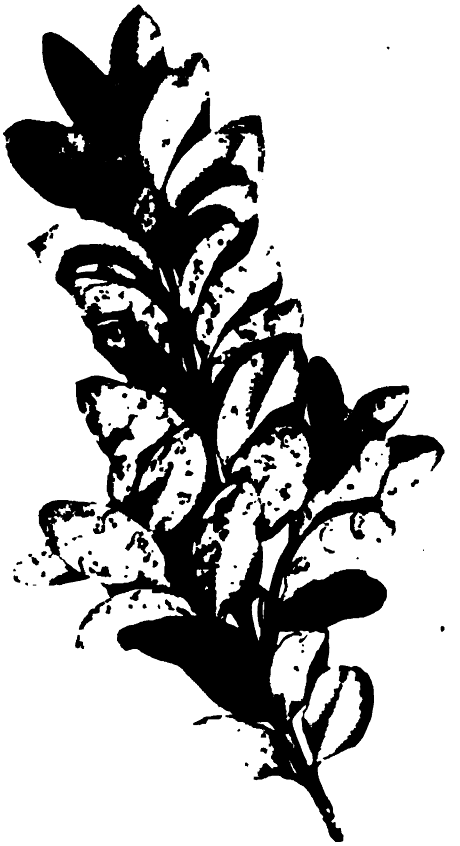

Fig. 2: Stark befallener Buchszweig nach dem Schlüpfen der Mücken. Die jungen Blätter sind schon wieder mit Eiern belegt.

hältnisse des Ortes bestimmt. Nach unseren Feststellungen in Dielsdorf und Engstringen im Jahre 1934, sowie in Luzern, Winterthur, Rafz und Dielsdorf im Frühjahr 1935, beginnt der Flug Mitte Mai etwa zwischen dem 10. und 20. des Monats. Je nach der Witterung ist mit einer Flugdauer von 2—4 Wochen zu rechnen. Die orange-gelben, schlanken Mücken verlassen die Blätter, ihre Geburtsstätte, indem sie sich mit grosser Mühe von den heraushängenden, durchsichtigen Puppenhüllen befreien (Fig. 1 und 2). Nach einer kurzen Ruhepause beginnt die Begattung und bald darauf die Eiablage. Ein Weibchen kann durchschnittlich 60—75 Eier ablegen. Alle diese wichtigen Lebensvorgänge spielen sich äusserst rasch ab, weil die Lebensdauer sehr kurz ist. Die Weibchen sterben nach 3—4. die Männchen schon nach 2 Tagen. Die Eier werden mit Hilfe eines kleinen Legestachels in das Blattgewebe auf der Blattunterseite gelegt. Bei starkem Befall sind pro Blatt nicht selten 10—15 Eier, oft noch mehr zu finden. Die Larven erreichen im ausgewachsenen Zustand eine Länge von 2—3 mm. Die Farbe ändert sich mit dem Alter; Junglarven sind weiss, spätere Stadien grünlich und zuletzt orange-gelb. Die Larven entwickeln sich in geschlossenen blasigen, flachen Minen im Blattgewebe. Die Minen oder Gallen sind im Juni als kleine, runde, dunkelgrüne Flecken auf der Epidermis der Blattunterseite zu bemerken; im Juli werden diese Flecken grösser, sind aber immer noch gleich gefärbt und getrennt: einige Wochen später laufen die blasigen Anschwellungen ineinander, färben sich gelblich-grün und sind auch auf der Blattoberseite sichtbar. Im Spätherbst ist die ganze Blattfläche blasig aufgeschwollen, die Gallen sind gelblich gefärbt. Die Larven überwintern in den Minen und sind gegen Kälte sehr widerstandsfähig. Die Verpuppung findet bei uns erst gegen Mitte April statt und die Puppenruhe dauert ca. 3 Wochen. Die Puppen sind 2—3 mm lang und an den dunkleren, seitlich gelegenen Flügelspitzen leicht erkennbar. Nach Ablösen der Epidermis auf der Blattunterseite sind die Larven und Puppen in den Minen leicht zu beobachten.

Durch die völlige Zerstörung des Blattparenchyms werden die Blätter vernichtet, trocknen nach dem Ausflug der Mücken aus und fallen ab. Die buschigen Buchskronen werden immer lichter, es folgt Triebdürre, Verunstaltung der Krone und nach wiederholtem, starkem Befall sogar Absterben der Pflanze.

Die Buchsgallmücke hat sich im Jahre 1934 stark vermehrt und ist im Frühjahr 1935 in manchen Gegenden verheerend aufgetreten; ganze Buchshecken sowie auch alleinstehende Pflanzen wurden stark beschädigt. Dank ihrer verborgenen Lebensweise hat die Buchsgallmücke wenig natürliche Feinde. Chaine konnte beobachten, dass Ameisen bei Beginn der Flugzeit den Mücken auf den Blättern nachgehen, diese aus der Puppenhülle herausreissen und wegtransportieren. Auch Vögel schnappen zahlreiche Mücken während der Schwärmzeit. Im Winter kommt es oft vor, dass die Meisen die Larven in den Minen entdecken; dann wird aber jedes Blatt fleissig untersucht, die Gallen werden aufgehackt und alle Larven herausgeholt. Die Unterseite der Blätter sieht ganz zerfetzt aus (Fig. 3). Diese natürlichen Feinde vermögen jedoch nicht, die grosse Zahl der Mücken zu vernichten und, wie wir gesehen haben, werden in sehr kurzer Zeit zahlreiche Blätter mit Eiern belegt. Es war deshalb wichtig, eine einfache, sichere und wirtschaftliche Bekämpfungsmethode auszuarbeiten.

Am einfachsten ist es, den Schädling an der Eiablage zu verhindern. Chaine hat beobachtet, dass die Mücken beschmutzte Blätter vermeiden und dass staubige Pflanzen viel weniger befallen werden. Man kann also durch eine rechtzeitige Bestäubung der Pflanzen mit einem feinen Pulver die Eiablage stark vermindern. Jedoch werden die ausschlüpfenden Mücken nicht vernichtet und finden immer wieder Blätter, um ihre Eier abzulegen, sodass der Erfolg nie vollständig ist. Anders aber, wenn die Pflanzen mit einem feinen Insektizidpulver, wie *Pirox*, bestäubt werden, und zwar kurz vor dem Ausschlüpfen der Mücken und einige Male während der Flugzeit. Die Tierchen, welche sich mit grosser Mühe aus den Puppen herausziehen und nachher auf dem Blatt ruhen, bis sie flugfähig werden, kommen sicher mit dem Pulver in Berührung und gehen zugrunde; diejenigen, die wegfliegen, kommen später bei einer Flugpause oder zur Eiablage auf die Blätter und werden ebenfalls getroffen. Wir haben in Luzern, Winterthur und Dielsdorf Bestäubungsversuche mit Pirox durchgeführt, die bewiesen haben, dass bei rechtzeitigem Eingreifen die Buchsgallmücke einfach und sicher zu bekämpfen ist. Ausschlaggebend für den Erfolg ist, dass im Moment, wo die ersten Mücken schlüpfen, die Blätter und besonders die Unterseite derselben mit einem feinen Piroxbelag bedeckt sind. Stäubt man erst, wenn schon zahlreiche Mücken sichtbar sind, so muss man damit rechnen, dass schon mehrere Blätter mit Eiern belegt sind. Bei uns muss man etwa um den *10. Mai* die befallenen Pflanzen untersuchen und bei Feststellung der ersten Mücken sofort stäuben.

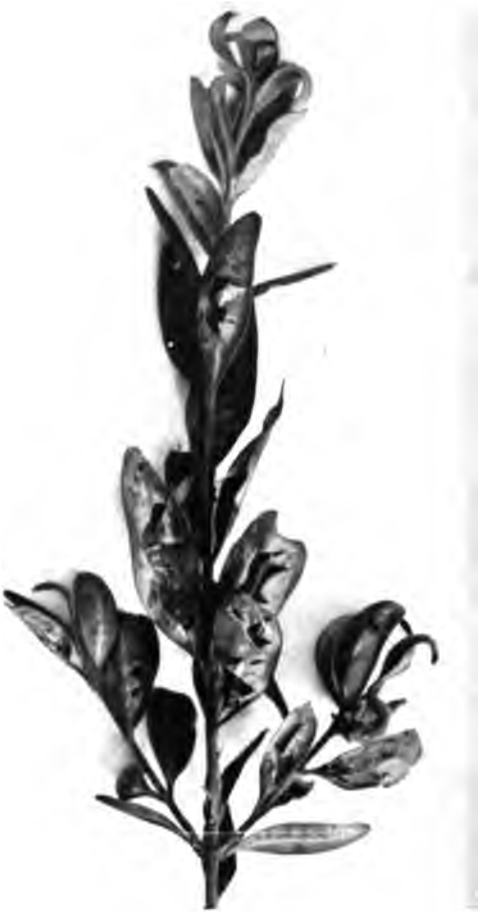

Fig. 3: Von Meisen zerfetzte Blätter durch Herauspicken der Mückenlarven.

Die sicherste Methode zur Bestimmung des Flugbeginnes ist die Kontrolle der Unterseite der Blätter. Sobald man vereinzelt Puppen beobachtet, welche aus den Gallen hervortreten oder wenn man schon einige heraushängenden leeren Puppenhüllen entdeckt, darf man nicht länger warten, denn bald folgt der Hauptflug. Die genaue Bestimmung dieses wichtigen biologischen Momentes ist ausschlaggebend für eine erfolgreiche Bekämpfung.

Die Bestäubungen müssen einigemale während der 2—3 wöchigen Flugzeit wiederholt werden, am sichersten alle 4—5 Tage, bei Regenwetter häufiger. Auch wiederholte Bespritzung mit Paramaag 2% zeigten ei sehr guten Erfolg. Durch die leichte V ölung der Blätter werden die Mücken an d Eiablage verhindert.

Stark befallene Pflanzen werden im F bruar zurückgeschnitten, damit sie im Frü jahr, nach der Behandlung mit Pirox, fris und üppig austreiben können.

Durch dieses einfache Verfahren hab wir die Möglichkeit, im Frühjahr zahlrei Buchskulturen, Buchshecken und solit Pflanzen von der schädlichen Mücke zu s bern und andere vor Befall zu schützen.

Dr. Ch. Hadorn, Entomologe der
Chemischen Fabrik Dr. Maag. Dielsd

Dr C. A. Kofoid
Compliments from
S Hadwen

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ASCARIASIS IN HORSES

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ASCARIASIS IN HORSES

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Ascariasis in horses seems to be common in Saskatchewan. Dr. B. H. Ransom and the writer (1918) performed autopsies on twenty-two horses in various parts of the province and found that twelve of these harbored *Ascaris equorum*. During the past season a number of infected animals have been found in the course of experiments on swamp fever. In 1923 seven cases of bronchitis and pneumonia in young foals developed at the University Farm at Saskatoon. A tentative diagnosis of ascariasis was made, but it was not possible to confirm it until later in the year, when the university veterinarian, Dr. Wright, treated these same animals with carbon bisulphide and oil of chenopodium. Several of the colts passed large numbers of ascarids and one of them over 300. Two of the colts died soon after the outbreak started, another lingered on but died some months later, the remainder coughed and ran at the nose but eventually recovered. The fact that no other horses at the university were suffering from colds at the time the colts became ill was the reason for the tentative diagnosis of ascariasis.

METHOD OF INFECTION IN FOALS

Ransom and his associates have shown the various ways in which pigs pick up Ascaris eggs. But in the case of horses there must inevitably be differences. Foals come into the world under much the same surroundings as pigs—that is to say, the mare is put to foal in a loose box. It is here no doubt that the young foal picks up many eggs—not so much during the act of suckling as is the case in pigs—but when nosing about among the litter on the floor of the box stall. Colts have a habit often of nibbling at manure, especially in the barn yard where the piles of dung might easily contain ripe eggs. Out in the fields it is not so probable that foals would pick up eggs—except from the afore-mentioned habit of nibbling at piles of droppings. As the eggs do not hatch on the ground they would tend to gravitate under the vegetation, helped by wind and rain, and there remain harmless. It appears likely thus that the box stall and the stable are the places to look to for the source of the infection. Thus the season of 1923, when the foals became infected, was wet, and the foals and their dams spent a good part of the early season in the stables. 1924 was a dry year, and they were not brought in as much; added to this the stables had been newly painted, so the 1924 crop of foals escaped infection so far as is known. The prevention of ascariasis would accordingly seem to be largely a matter of stable cleanliness.

It is believed by many that immunity to or tolerance of worms and other parasites increases with the age of the host, and that the mere fact of an animal getting older enables it to tolerate or resist them to a greater degree. An attempt will be made by the writer to explain some of his own observations on animals and to mention some of the views of other writers which bear on the subject. The damage parasitic worms produce comes under two headings, first mechanical, and second, that caused by their excretions or secretions. The mechanical part will not be dealt with here beyond pointing out that organs or tissues which have been invaded by parasites may have had their functions upset by nerve irritation caused by injury, or by hemorrhages and other means.

The products given off by worms cause their host to become sensitive to them. Many workers seem to agree on this point. The balance between these substances and the anti-substances which neutralize them must be very evenly distributed just as they are in some of the bacterial diseases. There is evidence that this is correct in that adult animals are commonly able to maintain their good health while harboring many parasites. Young animals not having yet developed this power of tolerance suffer severely from their effects. The dangerous time for young animals appears to be, especially in the case of Ascaris, when the migration takes place through the body. The passage of the larvae through the liver brings on a degeneration of the liver cells which must have a serious effect on nutrition. In the lungs edema follows their migration, which in turn may result in more serious disturbances. In the older animals, which on autopsy are found to be carriers of Ascaris, the liver and the lung lesions, except for old scars, etc., may be entirely absent. This seems to point to the fact that once the worms have completed their migration and have settled in the intestine they have reached a sort of neutral position as regards the health of their host. It is still possible that the absorption of their dejecta in the intestine might keep up the irritation in sensitized tissues such as the lungs.

To sum up, the writer believes that the result of a first invasion of worms in an unresisting host is to stimulate the production of both antisubstances and eosinophiles to neutralize their cast off products, and that in addition to this there must be a third substance, which is antagonistic to the worms themselves; this substance which paralyzes or kills them he believes is secreted by the eosinophiles that are found surrounding the dead worms in the tissues. This theory is supported by the fact that he has seen cases where only a percentage of the invading parasites died surrounded by eosinophiles, whereas in the same host other worms evaded the eosinophiles either because of their rapid movements, or because owing to their numbers the eosinophiles were not numerous enough to cope with them.

SENSITIZATION

A suggestion is made that the edema of the lung following Ascaris invasion may be, in some cases, in part or wholly due to sensitization. This would be most likely to happen when two or more invasions of parasites have occurred in the same host. In two cases in colts the lungs appeared to be free from bacteria as all culture tubes inoculated from them remained sterile, yet parts of the lungs were edematous. In experiments made with other parasites the writer has noticed that tissues which have once been attacked by parasites become sensitive (local sensitization) and react again when the juices of the same parasites are injected into the general circulation.

OPINIONS OF VARIOUS EXPERIMENTERS

Ransom (1924) says: "Its significance [sensitization] in relation to the toxic action of Ascaris in cases of infestation with the parasite remains to be determined." Weinberg and Julien (1911) say: "Ces faits nous autorisent, croyons nous, a penser que les chevaux infestés par un certain nombre de parasites [Ascarids] s'immunisent petit a petit contre l'action des produits secretés par ces parasites." Roubaud and Perard (1924) say: "Les auteurs estiment qu'il s'agit d'un fait d'immunisation consecutif a une ou plusieurs atteintes subies" [Refers to Hypoderma.] Hadwen and Fulton (1924) state: "It seems, therefore, that the immunity that develops in the older cattle is effective in the prevention of warbles."

Fairley (1919), according to Agersborg (1924) "showed that there is a definite relationship between the cellulo-humeral response in experimentally infected monkeys (infected with *Bilharzia haematobia* and *Bilharzia mansoni*), and the prognosis. In hyperinfected monkeys dying within a few weeks he found that there was a constant leukopenia, absence of eosinophilia, and a negative complement fixation reaction. In monkeys surviving the sixth week of infection there was constantly present an eosinophile leukocytosis associated with a positive serological reaction." Yokogawa (1923) says: "We do not know how to ascertain the essence of the virus of the Ascaris larvae, but as the accumulation of eosinophile cells is conspicuous wherever they happen to be found, it is easy to infer their virulence." Höppli (1923) infected a large number of small animals with embryonated eggs of *A. lumbricoides* and also with Belascaris and Toxascaris. He found that the tissues reacted only slightly against the first invading parasites, but a few days later he found that the neutrophiles, eosinophiles, and mononucleurs appeared in groups, in some cases surrounding the parasites. In the case of *A. lumbricoides* there was little tendency on the part of the tissues and cells to encapsulate the larvae. On the contrary he found many encapsulated larvae of Belascaris in the livers of dogs. Höppli makes no mention of the ages of the animals he was infecting, and it would seem likely that in the Belascaris experiments he was dealing, in some cases at least, with dogs which had developed a certain amount of immunity to ascarids. Höppli himself thought that there might be some secretion which would cause a tendency to encapsulation and says: "Eine sehr wesentliche Ursache für die Knotchenbildung bietet auch die, allerdings noch unvollkommen untersuchte Absonderung spezifischer Stoffe." Höppli confirms Ransom's observations on the migration of the larvae via the blood stream, and denies Yoshida's views.

THE EOSINOPHILE CELL AS A DESTROYER OF PARASITES

In the experiments on Ascaris described below it has been shown that the percentage of eosinophiles in the general circulation lowers after an infective dose of eggs has been given and that it rises again soon after the worms have finished their migration through the tissues. The writer in cooperation with Ransom (1919) showed that injections of various worm juices in horses produced this same lowering and elevation of the eosinophile count. The writer in 1916, 1918, 1922 and 1924 also drew attention to the fact that the eosinophile cell was a most important factor in the prevention of infestation with both Hypoderma and Oedemagena. Vallillo (1919) says that the increase of eosinophiles is not a constant symptom perhaps because the eosinophiles come out of the blood vessels toward the tissues. S. Sterling-Okuniewski (1924) says: "Dans la trichinose, il y a une éosinophilémie, celle-ci pouvant etre légère (9 à 14 p. 100) dans les cas sérieux, ou très élevée (50 p. 100) dans les cas légers."

OBSERVATIONS ON EOSINOPHILES MADE BY THE WRITER AT DIFFERENT TIMES AND WITH VARIOUS PARASITES

Local reactions in which eosinophiles are numerous are uncommon in young animals and are common in older animals. In the case of the penetration of the skin of cattle by Hypoderma, the reactions become more and more severe as the season advances. That eosinophiles actually do neutralize verminous toxins is supported by the fact that these cells will traverse intact mucous membranes when these have been bathed in verminous juices. Eosinophiles surround worms of all sorts when they become stationary in the tissues. Eosinophiles take on a phagocytic rôle when bacteria are bathed in verminous juices. (This was first shown by Weinberg and Seguin, 1914.) Eosinophiles act in a local or systemic way, when large or small amounts of worm juices are injected into the body. Eosinophiles do not appear at once in the case of local injections; an edematous swelling comes first which indicates that the body fluids, also cells such as the neutrophiles and mononuclears, play a part in the neutralization of the offending substance. The slowness in the production of the eosinophiles does not indicate that they are unnecessary for the successful neutralization of toxin or for the destruction of parasites. It may be interpreted to mean that the cells are only produced as occasion demands. There is a great difference between the neutrophile cell and the eosinophile, for instance, the former cell is in constant demand to act in the defense against bacteria—these however, are constantly attacking animals while worms do so irregularly. The percentage of eosinophiles in the circulation may be low or the cells absent altogether in severe cases of parasitism. In such cases the eosinophiles have been drawn out of the circulation and are to be found in large numbers around about the parasites themselves. When the proper percentage returns into the general circulation it means that the parasites have been overcome and that there is a surplus of eosinophiles. With regard to worms situated in such places as the lumen of the bowel or in the cavities of the body, this may not hold true, but this refers to parasites within the tissues.

Experiments with *Ascaris equorum*. Experiment I (on a young sucking colt 12 days old)

Day 1 (May 20, 1923) Colt given a petri plate of Ascaris eggs.
Day 7 Colt breathing hard and seemed distressed.
Day 7-13 Colt distinctly unwell but no cough.
Day 13 Given second dose of Ascaris eggs.
Day 16 Colt coughs a little. Temperature 102.5.
Day 16-20 Coughs continuously.
Day 20 Colt killed. *Post mortem:* Liver badly spotted. Lungs slightly affected in anterior lobes. Numerous Ascaris larvae found in air passages which contained a considerable amount of mucus. In the duodenum a number of ascarid larvae about 2 mm. in length.

COMMENTS ON EXPERIMENT 1

The colt developed a husky cough after the administration of the second dose of Ascaris eggs. Larvae were recovered from the lungs in great numbers. It is probable that the time it takes *A. equorum* larvae to make their journey from the intestines to the lungs and back again is similar to that of *A. lumbricoides* in the pig, as fairly well developed larvae were found in the duodenum twenty days after the first dose was given and seven days after the second dose.

Experiment II (on a 4-5 months old colt naturally infected with Ascarids)

Day 1 A few Ascaris eggs found in feces.

Day 2 Colt given drench of Ascaris eggs.

Day 20-24 Slight cough.

Day 32 Differential leucocyte count. Monos. 62.2, poly 24.8, eosins 12.5, mast 0.5.

Day 33 Given second dose of Ascaris eggs.

Day 35, differential leucocyte count. Monos, 58; poly, 35.5; eosins, 5.5; mast, 1.

Day 38, differential leucocyte count. Monos, 69.5; poly, 29; eosins, 1.5; mast, 0.

Day 45, differential leucocyte count. Monos, 48.5; poly, 44; eosins, 4.5; mast, 0.

Day, 50, differential leucocyte count. Monos, 57; poly, 35.33; eosins, 5.66; mast, 2.0.

Day 52, differential leucocyte count. Monos, 47.5; poly, 41; eosins, 11; mast, 0.5.

Day 54, differential leucocyte count. Monos, 34.7; poly, 49.7; eosins, 13.3; mast, 2.3.

Temperatures. Taken daily during experiment. No rise over 102.5.

Day 54 Colt killed. *Post Mortem:* Animal pot bellied, slight anemia, lungs spotted with translucent white nodules. Liver spotted and degenerated. Heart, endocarditis. Ascarids in intestine, 19 male and female and 7 smaller worms. 183 small larvae about 2-3 c.m. long and 203 larvae about 1.5 c.m. long.

Cultures. Inoculations made from liver and lungs remained sterile.

COMMENTS ON EXPERIMENT II

This animal was beyond the age for severe symptoms to set in after the administration of eggs. The only effect noticed was a slight transient cough. This case is the most interesting of the three, as there was a well marked disturbance in his blood when the second dose of eggs was given. It seems evident that the diminution of the eosinophiles in the general circulation was due to their being attracted to the liver and lungs to repel the new invasion of larvae. They again appeared in the circulation—even in increasing numbers 21 days later. In sections made from the lung and liver (colts Nos. 1 and 2) eosinophiles were found in large numbers. In the lungs many larvae had been destroyed in their migration and small pearly white nodules were plentiful just under the pleural covering. In the liver no larvae were demonstrated in sections.

PATHOLOGICAL EFFECT OF LARVAE MIGRATING THROUGH THE LIVER

The liver cells showed a grey granular degeneration and many of them stained badly, especially the nuclei. Surrounding Glisson's capsule eosinophiles were grouped like a ring, in many cases, and they were also to be found under the capsule of the liver. It seems evident that many larvae instead of traveling by the circulation follow the connective tissues along the vessels. The eosinophiles were not scattered uniformly throughout the liver, so that the groups of eosinophiles round about the vessels made a striking picture. The tissues surrounding the vessels were absolutely free from eosinophiles but in those cases where the eosinophiles encircled them, numbers could be found actually in the coats of the vessels. Possibly these cells were passing through the walls of the vessels to join the cells outside. In the blood itself inside the vessels very few eosinophiles were noted. From the above it seems certain that the eosinophiles only collected round about the hepatic vessels and ducts in those places where the worms had actually travelled. Ransom and Cram (1921) tapped the blood vessels in different parts of the body and found the larvae inside them. Their observations give absolute proof that the larvae—no doubt the majority—travel by way of the vessels.[1]

The writer's observations, he believes, show however that many larvae follow the connective tissues along the vessels. The cellular reaction shows that such is the case because it is not likely that the eosinophiles would group themselves round vessels through which the larvae had merely passed on their way to the heart and lungs. Stewart (1917, quoted by Ransom) thought that one likely route followed by the larvae was along the capillaries of the liver between the degenerated columns of liver cells. However the degeneration of the cells could not have occurred until after the passage of the first batch of invading larvae.

The part played by the eosinophiles in the neutralization of verminous toxins and in the destruction of larvae would necessarily be after the first invasion; this view entirely fits the case with regard to the progressive development of immunity against ascarids, and is borne out by the differential counts given above. The mechanism of defense by the eosinophiles is no doubt imperfect in the domesticated animals, because of the sudden unexpected number of worms invading the body. If the invasion occurred little by little it is probable that the immunity would keep pace with the invaders. An example of an unresisting host to parasitic infection is well illustrated by the case of a calf attacked by warble larvae. The period of attack is short and the invasion often large.

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[1] In a personal communication Ransom says: "If the larvae follow the connective tissues along the vessels they ought occasionally at least to be found there, especially since their progress would probably be slow along such a path." This objection is no doubt valid with regard to the migration of the larvae through the liver, as in all the sections made no dead larvae were encountered. In the case of the smaller vessels of the liver where by mechanical injury or through their secretions, the larvae could cause actual damage, the cellular reaction might be considered as an attempt at repair. But in the larger vessels the onward movement of the larvae would be much quicker and not likely to damage the walls. In the lung sections numerous dead larvae were found surrounded by a zone of eosinophiles. However, where long streaks of eosinophiles were noted between the lobes, the reaction could be interpreted as consecutive to the passage of the larvae."

PATHOLOGICAL EFFECT OF LARVAE IN THE LUNG

A line of eosinophiles ran under the covering of the lung, and other lines could be traced following the bands of connective tissues between the lobes. Whenever a larva had been successfully surrounded by eosinophiles, the dead larva could be seen in the center of the ring. In the surrounding zone the material appeared to be of an amorphous nature in which there were many eosinophiles and innumerable eosinophilic granules.

Experiment III. (Colt 12 days old given a petri plate of *Ascaris equorum* eggs)

Day 1 (Oct. 9, 1924). Temperature 102.3
Day 5 Temperature 104.2
Day 9 Cough began and continued until day 140
Day 17 Temperature 105
Day 18 Temperature 104.2
Day 19 Temperature 104
Day 21 Temperature 105.2
Day 22 Temperature 105.2
Day 23 Temperature 105.1
Day 24 Temperature 104.2
Day 25 Temperature 104.3
Day 26 Temperature 105.2
Day 27 Temperature 103.4
Day 53 Temperature 104
Day 54 Temperature 104
Day 55 Temperature 103.4

Day 71 Temperature 105.3. No Ascaris eggs in feces. Differential leucocyte count: Poly, 57; monos, 43. No eosinophiles found though hundreds of cells counted.

Day 71-190 No temperature over 103.

Day 138 Numerous Ascaris eggs in feces.

Day 145 Differential leucocyte count: Mono, 65; poly, 31; eosins, 3.7; mast, 0.3.

Day 154 Differential leucocyte count: Mono, 77.3; poly, 19.7; eosins, 2.5; mast, 0.5.

Day 190 Differential leucocyte count: Mono, 79.5; poly, 18.3; eosins, 2.2; mast, 0. Colt has not developed well, his mucous membranes are pale and his abdomen slightly pendulous.

Day 191 Colt killed. Just before the colt was killed it was found that his temperature had risen to 105. On autopsy nothing was found to justify this rise — which may have no significance as regards Ascaris.

Post Mortem: Body fairly well nourished. Mucous membranes pale. The abdominal cavity contained about 200 c c. of fluid. This had been in much larger amount previously, as all the viscera were covered wth short fibrinous threads (¼-½ inch). The liver was enlarged and spotted and some small calcified tracks were noted. Heart, slight endocarditis. Lungs spotted; a few small hepatized areas in anterior lobes. On the dorsal surfaces of the main lobes the blood vessels showed small varicosities and portions of the lung were spotted and congested. The small intestine had a hard and thickened feel, it contained much white mucus. Two hundred and sixty-seven adult living ascarids were taken throughout its length, measuring on an average 24 cm. long. In the cecum and colon 29 dead ascarids were found. In the cecum 5 adult *Strongylus vulgaris* were collected and in the stomach 27 bots (*Gastrophilus intestinalis*). The colt had been kept in a loose box from birth (September 28th) and had never been taken out of the stable until after the winter set in in November—hence the colt had acquired Strongyles and bots from its mother in the stable. Smears made from liver and lungs showed a few eosinophils and some eosinophilic granules. Cultures made from the liver and lungs remained sterile. In the intestinal mucus eosinophiles were scarce; this also was the case in Colt II.

COMMENTS ON EXPERIMENT III

Colt III, like Colt I, was young and free from worms when the Ascaris eggs were given. Nine days later the colt began to cough. Soon after this his temperature rose and on the 17th day reached

105 F. It remained high until the 26th day when it fell a little. On the 53rd day it rose to 104 and on the 71st day went up to 105.3. After the 71st day no temperatures of over 103 were recorded up to the 190th day. It seems probable that the high temperatures were the result of infection following the migration of the larvae, however, there does not seem to have been any lengthy infection either in the liver or lungs as cultures made from them remained sterile.

The differential counts show that there was an absence of eosinophiles in the circulation on the 71st day, but on day 145 when the colt was over four months old the eosinophiles were returning though not in great numbers and they were also present on days 150 and 190. The total absence and then return of the eosinophiles suggests that the colt was beginning to resist ascarids, and it seems likely that if one or more doses of eggs had been given the eosinophiles would have increased as in the case of Colt II. In this connection it is interesting to note that a number of dead ascarids were being passed out by the colt. The exact significance of the cause of death in the worms could not be ascertained.

SUMMARY OF EXPERIMENTS

Three colts, two new born and one older, were dosed with embryonated *Ascaris equorum* eggs. In the two younger animals a cough developed 16 and 9 days after the eggs were given. The older animal only developed a slight cold after 20 days. Ascaris larvae were recovered from the air tubes. The eosinophiles in the circulation became more numerous after a second dose of eggs.

In Colt III no eosinophiles could be found in the blood stream 71 days after the infective dose was given, but they appeared on the 145th day. This is suggestive of the slow development of immunity. In Colt II an eosinophilia was stimulated by a second dose of eggs.

Sections of the liver showed a granular degeneration, and accumulations of eosinophiles around Glisson's capsule. In the lung the eosinophiles were grouped under the pleura and between the lobes. This eosinophilia suggests that many of the larvae migrate along the connective tissues outside the vessels. Dead larvae were found in the lungs surrounded by eosinophiles.

The theory is advanced that immunity to ascarids is stimulated and increased by repeated attacks of these parasites. It is also suggested that in addition to the production of anti-substances to neutralize the cast off products of the worms, there is another substance secreted by the eosinophiles which is detrimental to the worms themselves.

ACKNOWLEDGMENT

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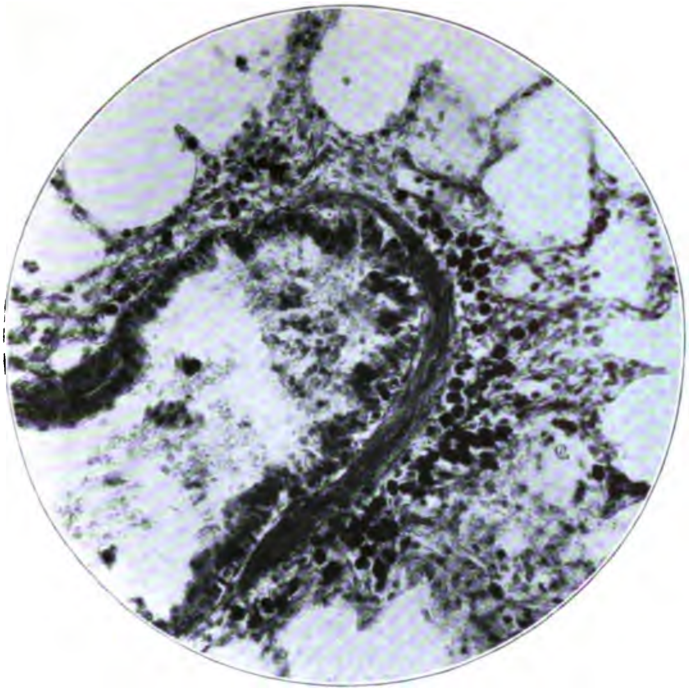

Figure 1

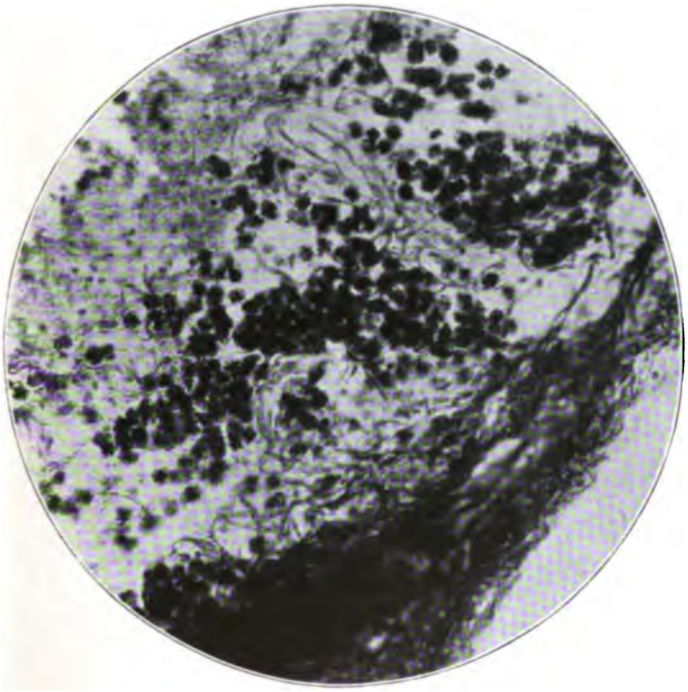

Figure 2

PLATE I

EXPLANATION OF PLATE I

Fig. 1.—Eosinophiles around air tube in lung, from colt II (Photomicrograph by A. Savage).

Fig. 2.—Eosinophiles grouped under covering of lung. Colt II (Photomicrograph by A. Savage).

THE

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17

A CONTRIBUTION TO THE KNOWLEDGE OF THE BOT-FLIES, GASTROPHILUS INTESTINALIS, DEG., G. HAEMORRHOIDALIS, L., AND G. NASALIS, L.

BY

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(PLATE II.)

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A CONTRIBUTION TO THE KNOWLEDGE OF THE BOT-FLIES, GASTROPHILUS INTESTINALIS, DEG., G. HAEMORRHOIDALIS, L., AND G. NASALIS, L.*

By S. Hadwen, D.V.S.
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(Plate II.)

The following is an account of an investigation into the life-histories and habits of the species of bot-flies (*Gastrophilus*) occurring in the western provinces of Canada. The investigation was undertaken co-operatively by the Entomological and Health of Animals Branches of the Dominion Department of Agriculture under the direction of the Dominion Entomologist and the Veterinary Director General. The greater proportion of the work, for which the authors are equally responsible, was carried out at the University of Saskatchewan, Saskatoon, Sask., during a period of about four months extending from June to September, 1917. Certain of the experiments dealing with the penetration of the larvae of *G. intestinalis* into animal tissue were commenced at Saskatoon, but were supplemented later in the same year by others carried out in the Department's laboratories at Agassiz, British Columbia.

Recognizing the scantiness of the knowledge pertaining to the early stages and habits of the three common species of bot-flies, the authors had in mind in this work the elucidation of several obscure points, more particularly relating to the hatching of the eggs, the form and behaviour of the first-stage larvae, the specific regions of the host selected by the different flies in ovipositing, and the manner in which the persistent attacks of the flies affect their hosts psychically.

When one considers how common these insects are, and that they have been known to both entomologists and veterinarians for several decades, it is surprising to find how inadequate is our knowledge of the species. Indeed, the theories regarding the hatching of the eggs and the mode of entrance of the larvae to the alimentary canal of the host have passed untested as genuine currency through the literature from the earliest times until quite recently, when the important discovery of the direct penetration of the skin of cattle by the ox-warble (*Hypoderma*), by Carpenter and Hewitt in Ireland (1) and one of the authors in Canada (2), has been almost paralleled by the significant results of Roubaud's investigations (3) in France. This last author observed in his experiments with guinea-pigs that, when the recently-emerged larva of *G. intestinalis* is brought into contact with the buccal mucosa, it promptly burrows into the mucosa and lies immediately beneath and parallel to the surface..

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The facts regarding the life-history and habits of *G. intestinalis* are much more extensive than those of *G. haemorrhoidalis* and *G. nasalis*, thanks, in great measure, to the excellent and careful work of Osborn (4), who has emphasized the importance of moisture, friction and warmth as factors necessary to the successful hatching of the eggs. His results in this direction have been in the main confirmed by Collinge (6).

The Egg.

Gastrophilus intestinalis.

The stalkless egg is generally laid at the distal end of a hair, in full view. It measures on the average about 1·25 mm. in length and about 0·379 mm. at its widest part just posterior to the operculum. General colour, greyish yellow to yellow. General shape navicular, more or less flatly oval in section. Two attaching sub-parallel flanges project on the side proximal to the supporting hair, which they encircle, commencing at a point removed about one-third from the anterior end of the egg and terminating just short of the posterior extremity; the non-flanged, anterior third makes an angle of about 30 degrees with the supporting hair. Chorion traversed by a close series of very delicate, transverse, sub-parallel striae continuous with those of the flanges; some striae interrupted and broken in their course. Operculum non-striate and apparently unsculptured, more or less flat, arising and arching somewhat abruptly on the side nearer the hair, imparting a rather truncate appearance to the anterior extremity of the egg (fig. 1). Chorion closely invested internally by a delicate membrane enclosing the larva (fig. 2).

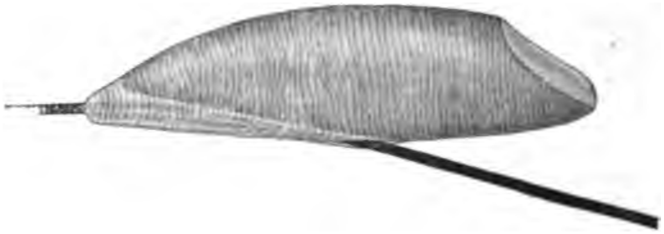

Fig. 1. Egg of *Gastrophilus intestinalis*, × 50.

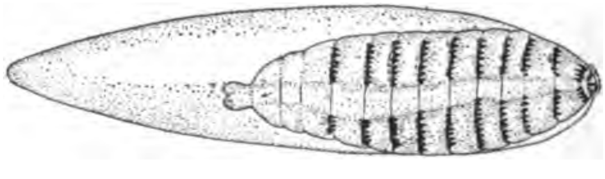

Fig. 2. Egg membrane enclosing unhatched larva of *Gastrophilus intestinalis*, × 70.

The eggs of *G. intestinalis* may be found promiscuously on the shoulders of the horse, mane, fore-legs, antero-interno-inferior part of the knee and postero-internal region of the fetlock. The long hairs which invest the inside of the fore-legs would appear to be especially favoured by the adults in ovipositing. The eggs have also been found on the hind-legs, particularly on the inside of the hocks, but here they occur only in small numbers.

In a paper by M. J. Rodhain (13), to which our attention was drawn by M. J. Bequaert, of the American Museum of Natural History, New York, the author records the finding of the eggs of the Oestrid of the rhinoceros (*Gyrostigma*), which were submitted to him from the Belgian Congo. In his description he says that the general form of the egg recalls that of *Gastrophilus intestinalis* by reason of the delicate and regular transverse striation of its walls, as well as by the oblique attachment of the apical operculum. It differs, however, from the egg of *G. intestinalis* in that it is devoid of flanges, their function being assumed by a posterior falciform appendage applied to the skin of the host. It is further suggested that the hatching of the eggs is effected in a manner similar to that which occurs in the case of *G. intestinalis*, namely, one animal licking another.

Gastrophilus nasalis.

Egg stalkless, generally laid at the proximal end of the hair near the skin, obscured externally among the overlapping hairs. Average length about 1·25 mm. Average breadth at widest part, midway between the two extremities, about 0·339 mm. General colour, whitish yellow. General shape elongate ovate, sub-circular in section. The two attaching sub-parallel flanges commence just posterior to the anterior extremity and run almost the entire length of the egg, stopping just short of the posterior extremity. The long axis of the egg almost parallel to that of the supporting hair. Chorion traversed by a close series of very delicate, transverse, sub-parallel striae, continued on the flanges; some striae interrupted and broken in their course. Operculum non-striate and non-reticulate, dome-shaped, its horizontal axis at right angles with the longitudinal axis of the egg, terminally placed on the anterior end (fig. 3). Chorion with internal investing membrane enveloping the unhatched larva.

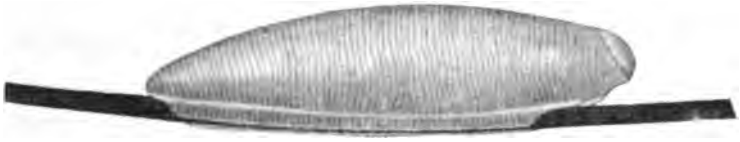

Fig. 3. Egg of *Gastrophilus nasalis*, × 50.

The eggs are generally laid on the hairs of the intermaxillary space between the rami of the mandibles beneath the head. Theobald (6) and other authors are of the impression that the species deposits its eggs in the nasal orifices, but in our experience, this is contrary to fact.

Gastrophilus haemorrhoidalis.

Egg stalked, the stalk a continuation of the broad chorionic flanges, hollow and open all along one side for the reception of the supporting hair. Average length 1·5 mm. Average breadth at widest part near the centre, 0·345 mm. General colour brownish black. General shape somewhat barnacle-like and compressed, flatly ellipsoidal in section. The two attaching sub-parallel flanges commence at a point removed about one-third from the anterior extremity of the egg, are continued into the stalk, which is about two-thirds the length of the egg, and taper off somewhat abruptly (fig. 4). The chorion encircled by a close series of fine striae, bearing on their anterior margins tiny, slightly recurving spinules anteriorly directed, the spinules of any one stria all but reaching the stria immediately anterior. Egg disposed parallel to the hair, with the exception of a slight outward deviation of the unflanged anterior portion. Operculum placed antero-externally, non-striate and non-reticulate, elongate-oval, slightly concave on its inner side (fig. 5).

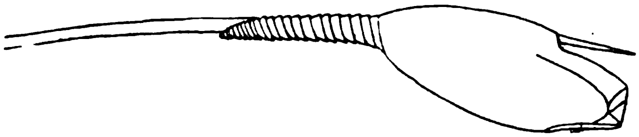

Fig. 4. Egg of *Gastrophilus haemorrhoidalis*; outline sketch to show attachment to hair and disposition of bands on stalk, × 65.

G. haemorrhoidalis generally lays its eggs on the hairs of either of the lips of the horse, but preferably on those of the lower. Parker (7), among other authors, states that the eggs are also found in the nostrils, but it has never been our experience to find them elsewhere than on the lips.

According to Parker (7), eggs of this species were found by himself and his assistant thrust into the nose and lips of horses, sometimes to the full length of the stalk. Thus he finds a reason for the nervous and occasionally uncontrollable fear shown by horses when the fly is "striking." Further, he states that the chitinous "bands" of the stalk impart to it the appearance of a screw, and that the structure is patently adapted to hold the egg in the skin after insertion. In no single instance did we find the egg penetrating the skin. On the contrary, we invariably found it adhering to a hair (fig. 4) and not touching the skin. Therefore, we conclude that Parker must have erred in his observations.

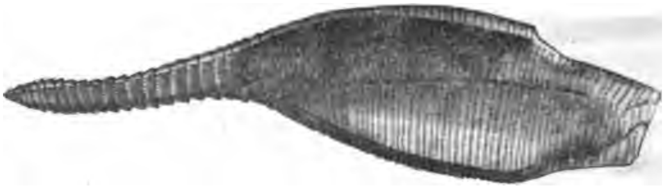

Fig. 5. Egg of *Gastrophilus haemorrhoidalis* removed from the hair to show the continuity of the flanges with the stalk, × 69.

The recently emerged Larva.

Gastrophilus intestinalis.

Number of segments 13, hairless. Length, 0·866 mm.; greatest width, 0·217 mm. General colour translucent white; general shape strap-like and fusiform, tapering towards both extremities. Unpaired, lance-like mouth-stylet (mandible) directed anteriorly. Anterior margin of the first segment with a few recurving, small, black hooks. Anterior margin of the second segment bearing an armament of long, recurving, black hooks (head-hooks), diminishing in size posteriorly, interspersed with small hook-shaped, placoid denticles. Posterior margins of segments 2 to 10 provided with an armature of irregularly-disposed rows of similar cuticular denticles and sub-ovate, brownish-black, flat scales, the denticles predominating in segments 2 to 5 and the scales in the following segments; segments 11, 12 and 13 devoid of armature. There is a slight medio-dorsal and medio-ventral interruption in the armature of each segment. Two divaricating, cylindrical, fleshy processes arise from the 13th segment, each process containing the prolongation of the main tracheal trunk of its side and bearing at its distal end the posterior spiracle (fig. 6).

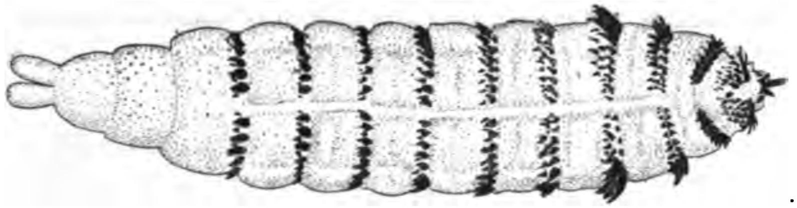

Fig 6. Larva of *Gastrophilus intestinalis*, × 120.

Raillet (8), on page 752, quotes Joly as asserting that spines similar to the crown of hooks of the head segment are present on the posterior margins of segments 2–10 inclusive, a view with which we entirely agree. In a footnote, however, this author quotes Brauer's opinion that these spines are more likely attached to the anterior margin of the segments following number 2, as is found in the mature larvae.

Gastrophilus nasalis.

Number of segments 12. Length, 0·829 mm.; greatest width, 0·116 mm. Segments 2 to 12 sparsely provided with regularly-arranged, slender, elongate hairs. General colour translucent white, with the exception of the black and brownish yellow of the hooks, denticles and scales of the segmental armature. Slender, paired mouth-stylets directed anteriorly from their articulation with the sclerites of the cephalo-pharyngeal apparatus. Anterior margin of first segment bearing an armament of recurved denticles, the ventral ones strongly developed, elongate and tapering gently to a slender, sharp, recurved point (head-hooks). Posterior margin of segment 2 provided with similar placoid denticles, but elongate ones lacking. Posterior

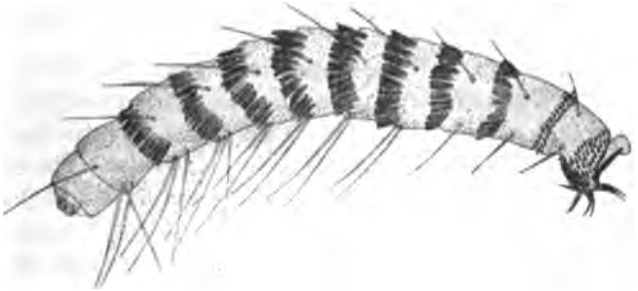

Fig. 7. Larva of *Gastrophilus nasalis*, × 114.

margins of segments 3 to 10 armed with narrow, elongate, sharp-pointed, denticular scales interspersed with similar smaller ones; base of scale brownish yellow, apices and margins black and more strongly chitinised; the larger scales apparently strengthened by a median rib. Segments 11 and 12 unarmed. Posterior pair of spiracles scarcely projecting beyond the extremity of the body, sessile (fig. 7).

Raillet (8), on page 758, and Neumann (9), on page 345, state that in the recently emerged larva of *G. nasalis* there is but one row of spines on the anterior border of the segments. With this opinion we do not agree, as examination of a large number of specimens shows that the spines or scales—as we prefer to call them—arise from the posterior margins, and further, their arrangement is somewhat irregular, so that they do not assuredly form a single well-defined row.

Gastrophilus haemorrhoidalis.

Number of segments, 13; hairless. Length, 0·585 mm.; greatest width, 0·133 mm. General colour translucent white, with the exception of the brownish-black of the denticles and scales of the segmental armature. General shape fusiform, tapering to both extremities. Single pair of mouth-stylets (mandibles) directed anteriorly. Strongly developed crown of elongate, slender, recurving head-hooks arising from the anterior margin of segment 2 with numerous smaller placoid denticles and toothed scales intermingled and irregularly arranged in a row. Segments 11, 12 and 13 devoid of armature.. Ultimate segment bearing posteriorly two cylindrical processes, at the extremity of each of which is a spiracle where the main tracheal trunk of each side terminates, as is the case in the larvae of *G. intestinalis*. The armature not so strongly developed as in the larvae of *G. intestinalis* and *G. nasalis* (fig. 8).

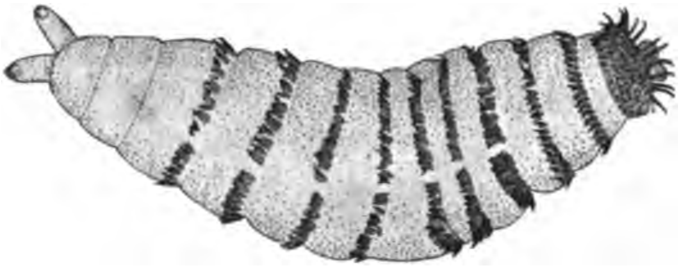

Fig. 8. Larva of *Gastrophilus haemorrhoidalis*, × 140.

In his description of the third-stage larva of this species, Neumann (9), on page 344, distinguishes it from *G. intestinalis* by its smaller size and its very deep red colour. The absence of spines on the mid-dorsal region of the ninth segment is mentioned and their complete absence on segments 10 and 11.

Hatching of the Eggs.

There seems to be some doubt as to whether the eggs of bot-flies require moisture and friction for their successful hatching. It has frequently been argued, notably by Osborn (4) and later confirmed by Collinge (5), that both moisture and friction are necessary factors in the hatching of the eggs of *G. intestinalis*. Whilst our experience would corroborate this statement, it can be definitely stated, as the results on Table I show, that the eggs of *G. nasalis* hatch quite freely without extraneous aid. For those of *G. haemorrhoidalis* no definite assertion can be made, as the number of eggs at our disposal was rather small. However, in one case, out of 14 eggs of this species, 8 hatched independently in a dry vial.

TABLE I.—*Experimental Hatching of Eggs of Bot-flies in Vials, unaided.*

Species.	Number of unhatched eggs.	Date when collected.	Number of eggs hatched.	Date of hatching of last egg.	Remarks.
G. intestinalis ..	7	July 28	0	—	3 alive Aug. 28, 4 dead.
	250	Aug. 5	1	Aug. 16	Larvae in eggs alive Aug. 16
	206	Aug. 20	4	Sept. 6	Remainder dead.
	13	Aug. 26	0	—	All dead in September.
	12	Aug. 27	0	—	Alive Sept. 4, died later.
	130	Aug. 27	6	Sept. 21	4 hatched only partly.
G. nasalis	40	July 11	32	July 23	Remainder died.
	69*	July 11	43	July 23	,, ,,
	29	July 18	22	July 28	,, ,,
	8	Aug. 3	0	—	Aug. 29, all dead.
	79†	Aug. 5	28	Aug. 20	Remainder died.
G. haemorrhoidalis ..	14	July 20	8	July 24	1 damaged, remainder died.

* The glass vial containing these eggs was kept slightly moist by means of a wetted piece of blotting-paper inserted between the cork and the walls of the vial.

† Includes some eggs which had hatched before they were collected from the horse, the number of which was not determined.

In the case of *G. intestinalis*, the experiments were continued until the end of September, and the eggs were frequently examined, at least three times a week. A large number of eggs in each batch were dissected after the date on which the last larva was observed to emerge. In most cases the larvae inside were alive and, when liberated, were quite active. From the results, it may be apparently deduced that the eggs of *G. intestinalis* normally require assistance in hatching in the shape of moisture and friction, and that only a very small percentage (1·7) contrive to hatch unaided. In support of this fact, it was found in frequent tests that hatching is readily promoted when the eggs are moistened and subjected to pressure by passing the blunt edge of a scalpel over them. In some, only slight pressure is required, just sufficient indeed, to cause the operculum to spring from its attachment with the egg. The length of time that moisture is applied may also determine the rate of hatching.*

From the foregoing table it would appear that the eggs of *G. nasalis* hatch most readily of the three species without artificial aid (51 per cent.), a fact which is more than interesting in view of the position where the eggs are deposited on the host, namely, the intermaxillary region. This also agrees with our theory that the larvae of *G. nasalis* may penetrate the integument of the host directly, since it is manifestly impossible that the animal could reach the eggs with its lips or tongue.

Actually, 51 is a low estimate of the percentage of hatching of *G. nasalis* eggs, for it does not take into account the fact that in the case of the batch of 79 eggs several had hatched before being collected.

It would be unwise to generalise from the results obtained in the case of the eggs of *G. haemorrhoidalis*, owing to the small number with which we dealt.

In passing, it may be observed that the eggs of *G. intestinalis* are disposed on the hairs differently from those of *G. nasalis*. On long hairs bearing a number, the eggs of the former are usually separated from each other more widely than are those of the latter, of which as many as 7 and 8 eggs are often deposited in close series, the anterior extremity of one in contact with the posterior extremity of its immediate neighbour. As already remarked, the eggs of *G. haemorrhoidalis* do not have the stalk touching the skin of the host, and generally there is but one egg on each hair.

Do the recently emerged Larvae of Bot-flies penetrate the Integument of the Host?

It is significant to note that about the time that the larvae of *G. nasalis* were hatching from the eggs in maximum numbers, numerous small scabby areas were observed in the intermaxillary region of the host. As to whether the cause could be attributed to the animal rubbing the affected part on fence-posts, is a moot point. In the animals examined, it could not be shown that the hair under the jaws had been rubbed off, as one would have naturally expected had the above been the reason. It is just possible that the newly-hatched larvae were the direct agents concerned in producing this abraded condition of the skin. Although we cannot substantiate this theory by material evidence, is it not permissible to suppose that, since it would be impossible for the host to lick the eggs with its tongue by reason of their inaccessibility, the young larvae, instead of being taken into the mouth, penetrate into the body directly through the integument ? This would undoubtedly account in satisfactory fashion for the small lesions which were noted. This theory receives additional support from the fact that in several of our experiments many eggs hatched independently, unaided by either moisture or friction, in a glass vial.

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* Since completing the manuscript of this paper, the authors have had the opportunity of consulting Bracy Clark's work (14) on the OESTRIDAE. In the hatching of the eggs of *G. intestinalis*, he states how he found that moisture and friction were necessary, and that these conditions were supplied when the host licked the eggs. As for the stimulus which causes the horse to lick the eggs, Clark advances the ingenious theory that the irritable biting of other flies, which he enumerates as *Oonopes*, *Tabani*, and *Muscae*, is responsible, and indeed, is of the opinion that the great majority of the larvae reach the mouth in this way. It is undoubtedly true that *Stomoxys* by its attacks will furnish this stimulus. The author also mentions the liability to infestation when one horse performs the friendly operation of licking the egg-infested coat of another.

As regards the eggs of *G. haemorrhoidalis*, Clark was the first to observe that they were deposited on the lips and that they were of a darker colour than those of *G. intestinalis* or *G. nasalis*.

Up to the time of appearance of Clark's paper the three species of *Gastrophilus* had been hopelessly confused with each other and with *Hypoderma*. The author rendered a material service to entomology by giving clear descriptions of the flies, accompanied by excellent illustrations, thus enabling one to discriminate the three species readily.

In reviewing the literature, it is interesting to note that Brumpt (10), p. 699, quoting Henry, says that the eggs of *G. nasalis* are laid on the cheeks and intermaxillary region of the horse and are entirely adherent to the hairs, a fact with which we readily agree. This same author, in a footnote, also quotes Raillet, on page 699, as stating that the first-stage larvae of this same species are probably the cause of " creeping myiasis " on the cheeks of the horse, indicating that he found a condition similar to that already noted in our observations.

Similar scabby pimples have been diagnosed on the lips of the horse, on the hairs of which the eggs of *G. haemorrhoidalis* are generally found. In this case, however, the eruptive condition might be satisfactorily explained, in the absence of approved evidence, by the host rubbing its lips violently on the ground. At the height of the season, the adult fly was frequently observed to goad its host into behaving thus, especially when the " striking " was persistently repeated. Further, the rubbing of the lips was severe enough to cause the complete removal of the hairs.

On 20th August 1917, a piece of skin, the hairs of which bore numerous eggs of *G. intestinalis*, was removed from the umbilical region of a foal recently killed. To prevent its decay, the skin was treated with bichloride of mercury. In all, only three eggs hatched unaided, one on 25th August and the remaining two on 29th August. The larvae which emerged from these three eggs died among the hairs and apparently made no effort to burrow beneath the skin. Results of a similar kind were obtained with the eggs of the same species when another piece of skin was taken from the antero-intero-inferior region of the knee of a horse which had recently died, and likewise treated with bichloride as before. No larvae emerged of their own accord from the eggs deposited on the investing hairs. Therefore, from these two casual experiments, no evidence was forthcoming that the recently emerged larvae of *G. intestinalis* may penetrate directly beneath the integument.

On 18th September, a small piece of the buccal mucosa was removed from the inside of the lower lip of a horse and placed in a petri dish. Recently emerged larvae were obtained from the eggs mentioned in the two previous experiments and hatched by the aid of moisture and friction. In all, 9 live larvae were placed on the skin, which was kept moist by the application at intervals of a few drops of normal salt solution. The experiment was commenced at 7.30 p.m. By means of the binocular microscope, some of the larvae were observed to be actively working their mouth-parts. The head-hooks were extended and opened out in a lateral direction. This behaviour was continued for a time. Finally, the mouth-hooks were embedded in the skin as if preparatory to the larvae tunnelling beneath the surface. At 10 p.m., they were still actively engaged in the work, but no single one had made any extensive headway. The most successful had not penetrated further than the second anterior segment. Nevertheless, the fact remains that the mouth-hooks were securely fixed, so much so, indeed, that quite a little force had to be exerted in detaching them. When examined the following morning (19th Sept.) all were found to be dead, probably from the effects of the salt solution. None had made any further progress in their efforts at entrance than was observed the previous night. The experiment was again repeated on 21st September, but on this occasion two pieces of mucosa with the attached muscles, taken from a horse's tongue were used. One of the pieces was papillated, the other unpapillated. In the case of the first, 12 newly-hatched larvae of *G. intestinalis* failed to effect an entrance, but out of 4 larvae on the unpapillated portion, one succeeded in becoming completely buried in 8 hours after the experiment was commenced. After 6 hours, only the terminal spiracle-bearing processes were showing, and these were finally lost to sight.

The success of this last experiment stimulated the authors to make a further attempt, which was done in the laboratories at Agassiz, B.C., later in the season. In all, four experiments were made on 22nd October, with larvae hatched from eggs collected at Duncan, Vancouver Island, on 15th October. The larvae readily emerged when the opercula of the eggs were detached with needles. In each case the mucosa of the tongue of a calf just killed was used. For the first, the portion selected was unpapillated, taken near the tip of the tongue; for the others, papillated.

Briefly stated, the results were as follows. In experiment No. 1, of 10 larvae placed on the skin at 12.45 p.m., 6 began burrowing and effected an entrance. In only one case did the larva succeed in completely burying itself, and this occurred 7 hours after the experiment commenced. In the other cases, only partial success attended their efforts, and the progress made varied considerably. For instance, after 6 hours, 3 larvae had 3, 5 and 7 segments respectively still revealed, and another after 7 hours, had still 4 segments visible.

In experiment No. 2, of 6 larvae, one burrowed completely beneath the surface in 2½ hours, in close proximity to a papilla. In experiment No. 3, of 5 larvae, 2 effected an entrance and, in each case, became completely buried in about 4 hours. A third was only partly successful, all the segments being hidden except the last three, in 1 hour and 40 minutes. No further progress was, however, achieved.

In experiment No. 4, of 6 larvae, 2 burrowed beneath the surface and disappeared in 2½ hours. In both cases they effected their entrance by means of the circumvallate fossa of a fungiform papilla. In this particular experiment, the larvae proved very restless, at times travelling aimlessly around, and at others, settling down and making an attempt to enter. Some appeared to be partly successful in their efforts, but after a while they would withdraw their mouth-hooks and wander around restlessly again. In the third case, in which entrance was successfully effected, 8 hours were occupied in the process, the larvae being finally oriented at a sharply oblique angle with the surface.

In summing up the results of these experiments, we do not wish to attach too much importance to their significance, although they are certainly suggestive. Granted the larva finds its way to the mouth of the host, there is apparently no reason why it should not penetrate the mucous tissues therein. Indeed, its structure is admirably adapted for this purpose, as is readily seen when one considers the piercing function of the mouth-hooks and the backwardly-directed segmental denticles and scales, which cannot fail to advance the efforts of the larva, once it has effected an entrance.

Since the completion of these experiments, we find that Roubaud (3) has demonstrated that a larva, hatched from eggs sharply brushed by the lips of a guinea-pig, immediately burrowed beneath the buccal mucosa in the animal's mouth and maintained itself parallel to the surface in travelling towards the throat. Its course was followed for a period of nine days, when it had then attained a size approximately equal to that of the larva just previous to the first moult. From this it is inferred that the infestation of the horse is produced when, for some reason or other, the animal rubs the eggs with the buccal membranes of its lips. The author goes on to state that in cases of "creeping myiasis" due to bot-larvae, entrance is not apparently made directly through the epiderm, but by way of the exposed mucous membranes of the eyelids and lips, or of some skin lesion. Regarding his statement that larvae are unsuccessful in penetrating the skin of the horse, wet or dry, and die very rapidly, the negative results in our own observations of the larvae on fresh skin removed from horses, bear him out.

Hall (12) has recently succeeded in obtaining some interesting results regarding incidental myiasis of bots in dogs. Bots, according to this author, have been found on a few occasions in the stomachs of dogs, the infestation probably arising from the eating of bot-infested stomachs of horses. In his experiments, 6 specimens each of *G. intestinalis* and *G. nasalis*, enclosed in gelatine capsules, were fed to each of two dogs. Three specimens of the former succeeded in establishing themselves for a period of 35 days, after which the animal was killed and examined. They were found to have settled in such diverse locations as the stomach, the caecum and the lower part of the small intestine. The specimens of *G. nasalis* did not succeed in establishing themselves, and none were found on post-mortem examination. Negative results were also obtained in another experiment with the larvae of *G. haemorrhoidalis*, although the same animal retained one of six larvae of *G. intestinalis*, fed at the same time, for as long as 13 days. The author quotes the parallel results of Raillet, who worked along similar lines.

It would be irrational to attach too much value to these experiments of Hall and Raillet. It has been our experience that the mature larva of bots will attach themselves very readily to animal tissue of various kinds, and it is only natural to suppose that they will endeavour to fix themselves temporarily to the walls of the alimentary canal of any strange mammalian host. The fact that several of the larvae in Hall's experiments were passed per anum soon after they were fed to the dogs, seems to us to prove that their unaccustomed environment did not suit them.

Distribution in Canada.

So far as our information goes, *G. intestinalis* occurs in Ontario, Alberta and Saskatchewan, as well as in British Columbia. It is more than probable that it is present throughout Canada wherever its host is found. *G. nasalis* occurs in the three western provinces, Saskatchewan, Alberta and British Columbia; while *G. haemorrhoidalis* has been taken in Manitoba, Saskatchewan and Alberta. In the opinion of F. C. Bishopp, of Dallas, Texas, who writes under the date of 12th February

1917, there is little doubt but that the last-named species will be found to occur in British Columbia, as it has been reported in neighbouring regions of the United States from Western Montana.

Professor W. Lochhead (11) quotes the opinions of two correspondents from Western Canada, one from Saskatchewan. It is the idea of the first that the "nose-flies" arrived in the western territory eight years ago and were first observed about a year after the importation of some horses from the United States, from which the parasites may have been obtained. The second states that the "nose-fly" (apparently referring to *G. haemorrhoidalis*) made its first appearance in Saskatchewan some ten or more years ago and supposes it to have been brought from the United States or Mexico.

Period of Adult Activity.

In the vicinity of Saskatoon, *G. nasalis* is on the wing from the middle of July until the middle of August, although a specimen taken by Mr. T. N. Willing, of Saskatoon, at Maple Creek, Sask., is dated 30th October 1903. Eggs of this species that were collected in the first days of August were found to have already hatched. Our first specimen of *G. haemorrhoidalis* was taken on 11th July 1917, and the last on 11th August. It is probable that the adult activities of these two species are seasonally contemporaneous. *G. intestinalis* makes its appearance somewhat later than the other two, the first capture having been made on 22nd July 1917, and the last on 22nd August. On the Pacific Coast, *G. intestinalis* is found on the wing much later in the season. On Vancouver Island, a specimen was taken on 5th October 1917, whilst on 3rd October several were observed in copula, the flies being then very numerous.

Habits of Adults.

The three species of *Gastrophilus* are most active on bright sunny days, attacking their hosts when the weather is warm. Wind has apparently but a slight restraining influence. All three are strong on the wing, although *G. intestinalis* is least rapid in its movements. As regards their effect on horses, it has been observed that the attacks of *G. nasalis* and *G. haemorrhoidalis* inspire the animals with more acute nervous apprehension than do those of *G. intestinalis*. Persistent attacks will drive horses that are grazing in the open almost frantic, and a mad stampede on a warm day is a fairly sure indication that bot-flies are in the immediate vicinity of the animals.

In an endeavour to prevent the attack of *G. nasalis* horses are often observed standing head to head, resting their jaws on each other's necks. By adopting this attitude the host can the better protect itself against the fly as it "strikes" upwards to oviposit between the rami of the mandibles. When in harness, the animals strive to ward off the fly by drawing the head back quickly towards the neck, but the action is apparently not rapid enough to prevent the insect accomplishing its purpose. An egg is generally left behind, securely fastened to a hair at each attack.

In darting at the lips, *G. haemorrhoidalis* will cause the animals to nod their heads violently, to strain them forward and to open their lips. On repeated annoyance they endeavour to rid themselves of the irritating pests by rubbing their lips vigorously on the ground or any convenient object. With this species, as with the last-mentioned, the adult makes its attacks insidiously from below and is often observed flying beneath the abdomen of the horse, near the ground, as if waiting for a favourable opportunity to push home the offensive. A Saskatchewan correspondent of Prof. Lochhead (11) relates how an adult of this species deposited an egg on the back of his hand, attaching it to a hair. He also recounts that on several occasions the flies have " struck " at the underside of the brim of his hat.

A second correspondent, also hailing from Saskatchewan, remarking on the haemorrhoidal bot-fly, observes how " it flies very swiftly back and forth, poises itself for a moment, as though to judge place and distance and then darting upward, stabs a black egg into the lip or chin of the horse, and retreats as swiftly, only to return at the next suitable moment. The horse can hear it and awaits the attack with nervous apprehension. On feeling the needle-like thrust, it starts violently and rubs its lips or nose on the grass or against another horse." As we have already pointed out, the idea that the egg is inserted into the lip is a fallacy, and the actual deposition of the egg does not cause the animal any physical pain.

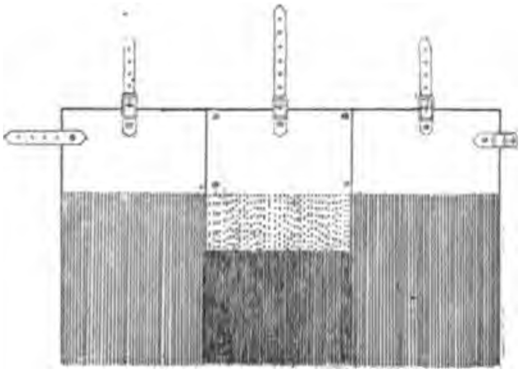

Fig. 9. Leather nose-fringe, extended, for protecting lips of horse against the attacks of *Gastrophilus haemorrhoidalis*, with central flap and fringe to cover the exposed upper lip.

The adult *G. intestinalis* does not apparently inspire such terror in the animals as the other two species. In thrusting home its attack, it may be often observed to hang on the wing, both before and after the operation is completed.

Preventive Measures.

It is usual for western farmers to shield the lips of their horses from the haemorrhoidal bot-fly by means of a wire-screen muzzle of fairly close mesh. Whilst quite effective for the purpose, it is rather clumsy, besides being an impediment to natural and free respiration. In order to obviate this difficulty, a device such as is represented in figures 9 and 10 might be contrived. The principle consists in nonplussing the attack of the fly by disturbing effects produced by hanging leather strips protecting the nose and lips and agitated into motion whenever the animal moves its head.

The apparatus, as devised by the authors with the aid of Mr. C. Warburton Young, is quite simple, consisting of a leather-band cut into a series of strips, each about three-sixteenths of an inch wide and long enough to cover the lips of the horse. The band is fastened by a buckle under the chin and attached to the head-stall by a similar buckle on each side and one on the front, as shown in figure 10. In order to eliminate the possibility of the nose being unprotected when the strips fall away too sharply as the animal raises its head, it is recommended that an extra leather-flap, as figured, should be attached to the band directly over the nose and likewise cut into strips on the lower margin. To secure a more rapid attachment of the nose-band to the head-stall, spring-snaps might be used in place of straps and buckles.

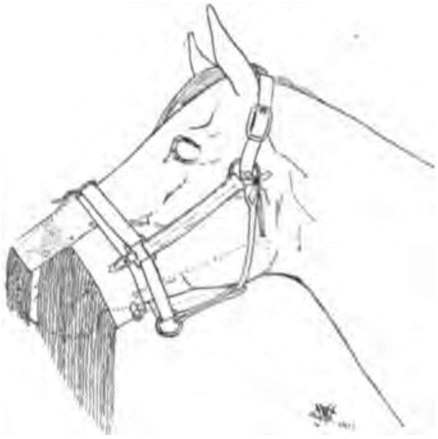

Fig. 10. Leather nose-fringe as it should be adjusted on the horse's nose. Dotted lines beneath the jaws indicate the outline of the canvas flap, attached to the head-stall and nose-fringe, for warding off the attack of *Gastrophilus nasalis*.

As a preventive measure against the attacks of *G. nasalis*, a piece of canvas extending from the nose-band to the throat is attached under the jaws. It is riveted to the posterior margin of the nose-band and fixed posteriorly to the ring of the head-stall on each side by a stout cord. The inter-maxillary region is thus completely protected.

Summary.

The eggs of the three species of bot-flies discussed in this paper are distinguished by the fact of that of *G. haemorrhoidalis* being the only one stalked. It is also longer than those of the other two species, which are of about equal length. Further, it is brownish black in colour, that of *G. intestinalis* being whitish yellow and *G. nasalis* yellow. The egg of *G. intestinalis* adheres to the hair by clasping flanges, which run only two-thirds of its length, whilst the flanges of the *G. nasalis* egg run almost the entire length.

The egg of *G. haemorrhoidalis* is not inserted nor screwed into the skin of the host. The eggs of *G. intestinalis* are laid indiscriminately on the body of the host, but preferably on the long hairs investing the inside of the foreleg. *G. nasalis* lays its eggs on the hairs of the intermaxillary space, and *G. haemorrhoidalis* on the hairs of the lips, preferably the lower.

Of the recently emerged larvae, that of *G. intestinalis* is largest in size and *G. haemorrhoidalis* smallest. In these two species there are 13 body segments, whilst *G. nasalis* has but 12 and is the only one bearing slender, elongate hairs. The larval posterior spiracles of the latter species are sessile; whereas in the others the two spiracles are borne on the distal ends of two cylindrical processes arising from the ultimate abdominal segments.

The eggs of *G. intestinalis* do not readily hatch unaided, but apparently require the application of moisture and friction or shock. A large number of *nasalis* eggs hatched spontaneously and a few of the *haemorrhoidalis* eggs also. This latter fact is regarded as supporting the theory that the newly emerged larvae of these two species may penetrate directly into the integument of the host. The lesions on the skin of the intermaxillary space and lips of the host observed at the time the eggs were hatching may be due to direct penetration of the larvae of *nasalis* and *haemorrhoidalis* respectively.

The newly emerged larvae of *intestinalis* failed to penetrate the hair-bearing integument of the host, but positive results were obtained when they were placed on portions of the buccal mucosa of a horse and calf recently killed. A larger number succeeded in penetrating the papillated portion of the calf's tongue, as compared with the unpapillated.

The three species are probably present in each of the western provinces of Canada.

As regards their seasonal appearance, *intestinalis* is somewhat later than *nasalis* and *haemorrhoidalis*, which appear simultaneously and are on the wing for about the same time. *G. intestinalis* continues to be active far into the autumn.

Of the three species, *intestinalis* causes the animal less apprehension than the other two.

The provision of leather-flaps on the lips of the horse, cut into strips, comb-wise, is advocated as likely to give good results in warding off the attack of *G. haemorrhoidalis*.

Acknowledgments.

The authors desire to render their grateful thanks to Dr. W. C. Murray, President of the University of Saskatchewan, for the laboratory accommodation which he so kindly granted. They are also indebted to Mr. T. N. Willing, Professor of Natural History in the University, for his generously allowing them to consult the specimens of Oestridae in his collection.

Ottawa, 28th November 1917.

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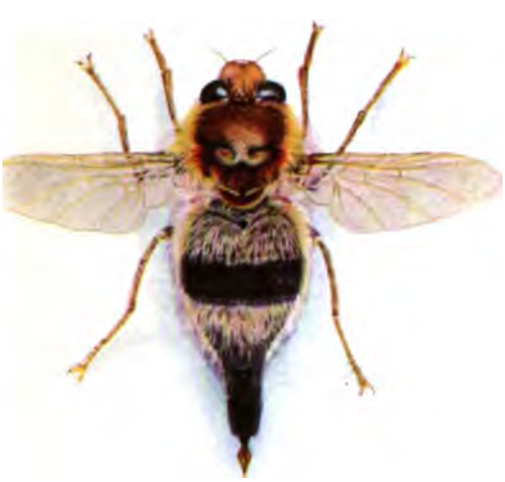
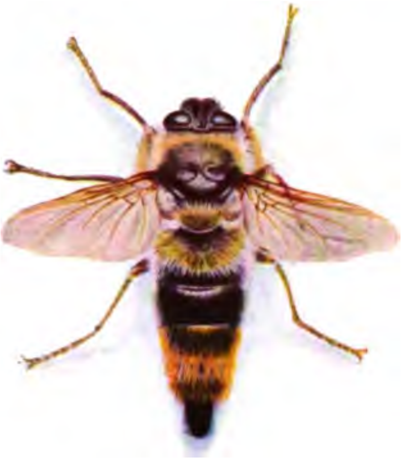
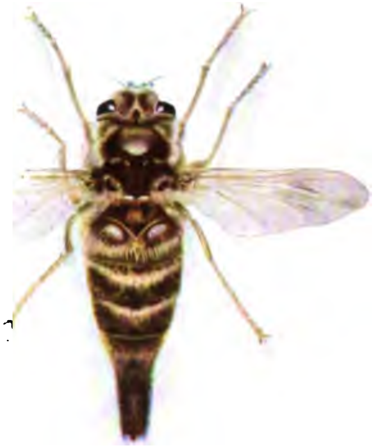


1. *Gastrophilus nasalis*, L. ♀



2. *Gastrophilus haemorrhoidalis*, L. ♀



3. *Gastrophilus intestinalis*, de G. ♀

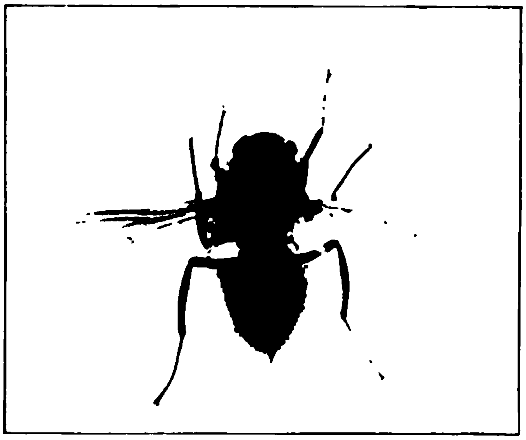
CANADIAN BOT-FLIES.

18/

DEPARTMENT OF AGRICULTURE
CANADA
HEALTH OF ANIMALS BRANCH

BULLETIN No. 16

WARBLE FLIES



THE ECONOMIC ASPECT
AND
A CONTRIBUTION ON THE BIOLOGY

BY
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THE ECONOMIC ASPECT OF WARBLE FLIES

By Seymour Hadwen.

During the summer of 1912 while conducting an investigation on Hæmaturia (Red Water) in cattle at Agassiz, British Columbia, I had an exceptional opportunity for studying warbles in the experimental animals under observation. When discussing my work with Dr. F. Torrance, Veterinary Director General, under whose authorization the work was done, he suggested that it would be well to find out what damage was caused by warble flies throughout the country, and with that end in view to communicate with the tanners in the different provinces. A circular letter was sent out from the head office and as a result thirty-six letters were received in answer. These letters have been abstracted and the results are tabulated below.

The evidence collected shows that warbles damage hides to a very great extent in the Dominion; it also clearly demonstrates that the investigation which has been conducted in a small way at Agassiz should be continued and extended.

There is no doubt that the abstracted information is most accurate, the letters coming from a class of sound business men, who have no interest in the matter save to get hides free from warbles.

The man who undoubtedly loses is the farmer; the tanner does not want warbled hides at any price, and several of them testify that they only buy hides during the season when hides are not grubby (the autumn).

The uses a grubby hide can be put to are very limited, and for some purposes appear to be useless; for instance in the manufacture of belts for machinery.

Several interesting facts are brought out concerning the grades of hides most affected with warbles, some of these can be explained by a knowledge of the life history of the parasite. Another point made clear is with a little care on the part of the farmer, certain classes of cattle would be kept comparatively free from warbles.

The class of animals which are the worst affected are 'range' cattle and here lies the most difficult problem, as no method of any sort has been found to prevent the ravages of the fly among semi-wild animals.

The economic loss caused by worry, loss of milk, etc., has not been dealt with, as no new facts have been ascertained.

STATISTICS COLLECTED FROM THIRTY-SIX LETTERS RECEIVED FROM CANADIAN TANNERS AND HIDE DEALERS IN ANSWER TO CIRCULAR OF VETERINARY DIRECTOR GENERAL. THREE LETTERS FROM NEW BRUNSWICK, TWO FROM NOVA SCOTIA, FOURTEEN FROM QUEBEC, SIXTEEN FROM ONTARIO, AND ONE FROM BRITISH COLUMBIA.

PERCENTAGE OF GRUBBY HIDES IN WARBLE SEASON.

New Brunswick.....	Tanner No.	1	75% in Grubby	Season.	75%	75%
Nova Scotia........	"	1	55 to 65%	"	60%	60%
Quebec............	"	9	75 "	"	75%	
	"	11	33 to 50%	"	41·50%	
	"	13	25%	"	25%	47·16%
Ontario............	"	2	30 to 40%	"	35%	
	"	4	50%	"	50%	
	"	5	50%	"	50%	
	"	6	25 to 75%	"	50%	
	"	7	25%	"	25%	
	"	9	50 to 60%	"	55%	
	"	12	25 to 75%	"	50%	
	"	14	25 to 50%	"	37·50%	44·06%

Average percentage for the four provinces........................ 56·55%

ANNUAL PERCENTAGE OF GRUBBY HIDES.

New Brunswick.....................	Tanner No.	1	35%		
	"	2	40%	37·50% Annually.	
Quebec...........................	"	1	25%		
	"	6	35%		
	"	14	12·50%	(10 to 15)	
	"	13	25%	24·37 Annually.	
Ontario...........................	"	8	50%	(one letter only)	50%
British Columbia....................	"	1	25%	25%	

Average percentage for the four provinces........................34·22%

This figure may be somewhat high and should probably be nearer to the one given for the percentage of annual damage to hides.

NOTE: The information abstracted from the letters is given in full, so that a better idea may be gained as to the value of the averages.

PERCENTAGE OF ANNUAL DAMAGE TO HIDES.

New Brunswick	Tanner No. 1	25 to 30% loss to all hides during season.		22·50%
	" 2	10 to 25 25%, no time specified.		
Quebec	" 6	Annual damage to hides	12·50%	12·50%
Ontario	" 5	" " "	25%	
	" 9	" " "	10%	
	" 12	" " "	17·50%	
	" 13	" " "	25%	
	" 15	" " "	10·20%	15%
				18·5%
British Columbia	" 1	" " "	25%	25%

Average annual percentage.................................... 19·62%

DAMAGE DONE TO HIDES BY WARBLES EXPRESSED IN GRADES.

New Brunswick	Tanner No. 2	Warbly hides classed as No. 2 or 3 grade.
Quebec	" 2	1 to 3 warbles in hide classed as No. 1. 3-12. No. 2 grade. Badly warbled classified as No. 3.
	" 3	Healed scars if large make No. 2.
	" 4	Healed scars make No. 2 or No. 3 quality.
	" 5	Warbles make No. 2 quality.
	" 7	Deduction on warbly hides 1 to 2c. per pound.
	" 8	Warbles cause loss of 50c. per hide all through year.
	" 9	Warbly hides damaged to the extent of 50c.
	" 10	Warbles damage finished hide 3–4c. per pound.
	" 11	Warbles damage hide 1 to 2c. per pound.
	" 12	Warbles damage hide 1 to 3c. per pound.
	" 14	Warbles damage hide 1 to 3c. per pound.
Ontario	" 1	Fifty thousand dollars annual loss to Canada through warbles.
	" 2	Loss through warbles 50c. to $1 per hide.
	" 4	Depreciation in value of hide 2–6c. per pound.
	" 6	Grubby hides classed No. 2 or 3 quality. to 2c. per pound loss.
	" 9	10% loss per annum i.e. 60c. per hide. Out of 300,000 hides the loss was $180,000.00.
	" 10	Grubby hides classed No. 2 or 3, loss 1 to 3c. per pound.
	" 11	1 to 3 warbles classed No. 1, over 3, No. 2 and 3 grades.
	" 16	2 to 5 warbles 1c. per pound, that is 60c. per hide. 5 or more 2c. per pound.
British Columbia	" 1	1/3 off for 5 or more grubs.

MONTHS IN THE YEAR DURING WHICH HIDES ARE MOST WARBLY.

New Brunswick....	Tanner No.	1	Early February to late July.
Nova Scotia........	“	2	First of March to latter end of July.
Quebec............	“	3	Latter part of December to early June.
	“	4	Worst March, April and May.
	“	6	January, February, March and April worst.
	“	9	February, March and April worst.
	“	11	April, May and June, slightly in July.
	“	12	February, March, April and May.
Ontario............	“	2	April, May and June.
	“	4	January to June.
	“	5	First of February to first of June.
	“	6	Middle of January to end of June.
	“	9	First of April to 15th of June.
	“	10	February to early July.
	“	12	February to June.
	“	15	Affected late October to early May, worst January to April.
	“	16	March to June.
British Columbia.....	“	1	January 1 to June 1.

The length of the warble season from the tanners' point of view extends from late January to early July, the worst period being during the month of April. From my notes made at Agassiz I find that the first larvæ emerged on April 10, and that the last were ready to come out on July 2; so that the table tallies absolutely with what I have found. The larvæ were naturally very prominent under the hides before April 10 and had perforated the skin some time before.

Nearly all tanners are agreed that the rough, long-haired, ill-kept animals are most warbly and that on the other hand well-fed sleek animals are not so badly affected. Of course weak animals are always more parasitized than the strong, and cannot fight the fly as well as the more robust; but I believe that it simply means that they are at the mercy of the fly all day long, whereas dairy cattle and well-bred animals are often housed during the heat of the day. Another reason is that cattle kept in or near towns will naturally be less exposed to the attacks of the fly, as there will be fewer about. Reasons for this are given later. Some of the tanners mention the fact that a wet season is beneficial in keeping down the number of grubs. The answer to this is simple. Warble flies are never seen in cold or cloudy weather. The hides coming from certain districts are mentioned as being comparatively free from grubs, especially in Ontario. I am not familiar with all the places mentioned, but some of them I know are dairy centres, and the above arguments will apply to these.

PLATE I.

WARBLE HIDES.

FIG I.

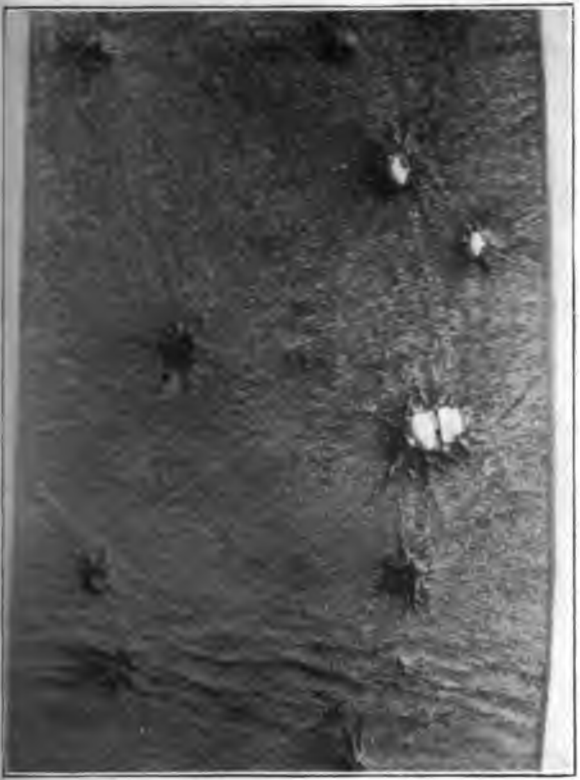


FIG. I.—Actual size of leather, 3¼ in. x 3¾ in.

FIG. II.

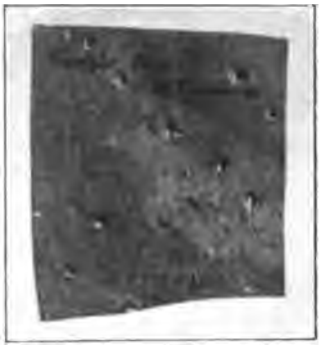


FIG. II.—Actual size of leather, 2¾ in. x 1¾ in.

A CONTRIBUTION ON THE BIOLOGY OF HYPODERMA BOVIS (DE GEER).

By SEYMOUR HADWEN.

The species of Warble-fly which is recorded here for the first time in Canada, and probably for North America, is *Hypoderma bovis* (De Geer).

Seeing that numbers of cattle are being imported yearly from Europe, it is strange that there seem to be no records of any previous captures, and in consequence no work has been done previously in Canada on the life history of this destructive insect. Owing to the kindness of Professor Carpenter of Dublin who sent me a specimen of *H. bovis*, I have been able to confirm the determinations I had made by descriptions alone. To show the rarity of these insects in entomological collections, I may mention the fact that none are to be found in the Departmental collection at Ottawa, and that a request for a specimen of *H. lineata* from the bureau of Entomology at Washington, D.C., could not be granted owing to scarcity of specimens. My thanks are due to Dr. L. O. Howard for specimens of the larvæ of *lineata*, which enabled me to still further confirm my opinion that I was dealing with *H. bovis*. Whether *H. lineata* is the common form in Canada I do not know, but it should not be a difficult matter to decide, as accurate determinations can be made by the aid of the spiny armature of the larvæ itself, and these at any rate are common. For the benefit of those who are not cognizant with the previous works on these insects, I may say that up to now *Hypoderma lineata* has been considered to be the Warble-fly of North America. All the observations recorded in this paper at Agassiz refer to *H. bovis.*

I will not go into many details or describe the fly fully, as I have prepared a coloured drawing; suffice it so say that compared with *H. lineata*, *H. bovis* is a more robust insect, and has yellow coloured hairs in the anterior part of the thorax (at the back of the head). The central part of the thorax is black and shiny. In *H. lineata* the thorax is nearly entirely covered with whitish and black hairs, except for the lines from which it gets its name; in *H. bovis* the tail end is covered with orange yellow hairs, in *H. lineata* the hairs are lemon yellow.

Through the courtesy of Mr. Ruggles of the Minnesota Agricultural College, I examined the collections there and found two females of *H. lineata* which compared with the above descriptions.

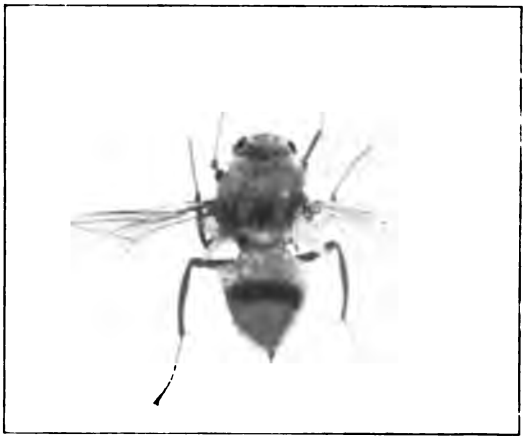


PLATE 2.

FIG. 1.

FIG. I.—Hypoderma bovis (De. Geer) °

(See also Frontispiece).

Photo S. Hadwen

Table Showing Average Time for Emergence of Flies from Pupa.

			Days.
May	7 Larva pupated.	Fly emerged June 16	40
	19 Larva pupated.	Fly emerged June 20	32
	20 Larva pupated.	Fly emerged June 23	34
	27 Larva pupated.	Fly emerged July 2	36
	28 Larva pupated.	Fly emerged July 2	35
June	5 Larva pupated.	Fly emerged July 11	36
	19 Larva pupated.	Fly emerged July 19	30
		Average number of days	34·7

The first fly captured out of doors was on June 8. Six in all were caught outside. The season was very wet, and as warble flies only appear in the finest weather, observations could only be conducted on those days. The last time cattle were seen to gad was on August 2, on which date flies were captured. On looking up the weather record, I find that there were 5·95 inches of rain in June, 5·09 in July, and 7·84 in August; there were 39 days, on which it rained, and of course many cloudy days, so that it seems fairly safe to predict that in this section of the country, warbles will not be as plentiful as usual next spring.

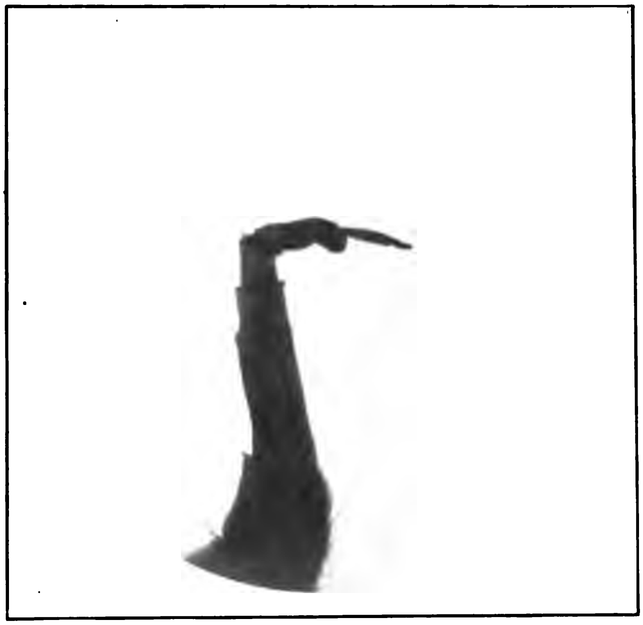
Experiments on Oviposition.

On June 19, a Warble fly was caught, and its wings clipped; the fly was put on to a cow's back. The ovipositor was thrust out several times and a white, milky substance deposited. After a time an egg was extruded, it was quite loose, but was pushed under the hairs. No more eggs were laid.

Another fly was captured, and its wings clipped, it did not try to oviposit, but kept feeling round with its ovipositor, and a milky substance was deposited several times. As the fly would not lay, a little pressure was applied to the abdomen, and an egg expelled. The fly was let loose again on the cow's back, where it shortly afterwards laid an egg; it was loose, and not attached to a hair.

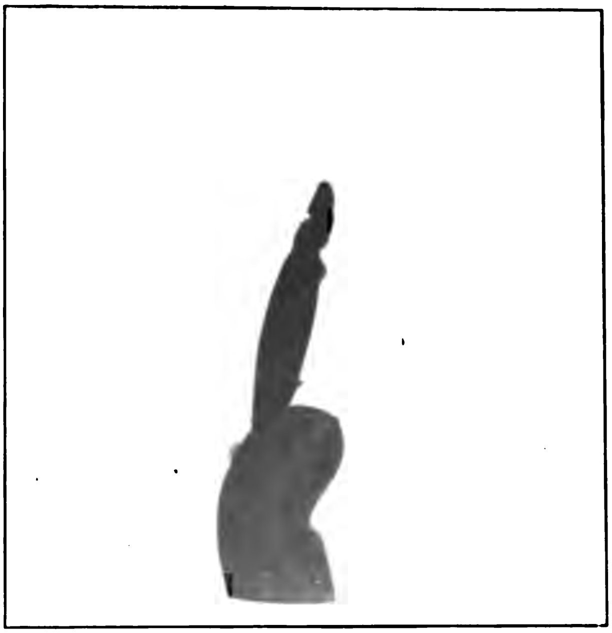
Both flies were put into a vial together, they began at once to tumble over one another, they extended their ovipositors at intervals, in all they laid twenty-five eggs, no milky substance was extruded. This dry method of egg laying, if one may call it so, seems to be peculiar, as will be seen later, for when a fly deposits an egg in nature on a hair, it is cemented on firmly. These eggs did not seem to be sticky at all, but the grooved end of the egg was closed, which may account for it. To ascertain more about this, pressure was applied to the abdomen of a female and the ovipositor extruded, when an egg slowly appeared. As soon as it was partly out, the ovipositor was cut off, and mounted on a slide. The egg comes out of the ovipositor with the grooved end first; so that the ovipositor must in some way open out the groove of the egg, adjust it to a hair, surrounding it with the gummy substance before it fully comes out. I do not mean that the gummy substance is necessarily secreted at the time the egg is laid, but probably what happens is this:—the groove is opened and fitted on to a hair, and a certain amount of the gummy material within the groove displaced by the hair; this accounts for the surplus found at the point of attachment. The accompanying photographs illustrate this.

Fig. I PLATE 3.



Ovipositor with egg.

Fig. II. PLATE 3.



Tip of ovipositor and egg enlarged. Photo S. Hadwen.

One peculiarity worth noting and which I have not seen mentioned before is that the egg is attached to the base of the hair, it was often quite difficult to pass a thin pair of scissors between it and the skin. The accompanying photographs also show this.

FIG. I. PLATE 4.

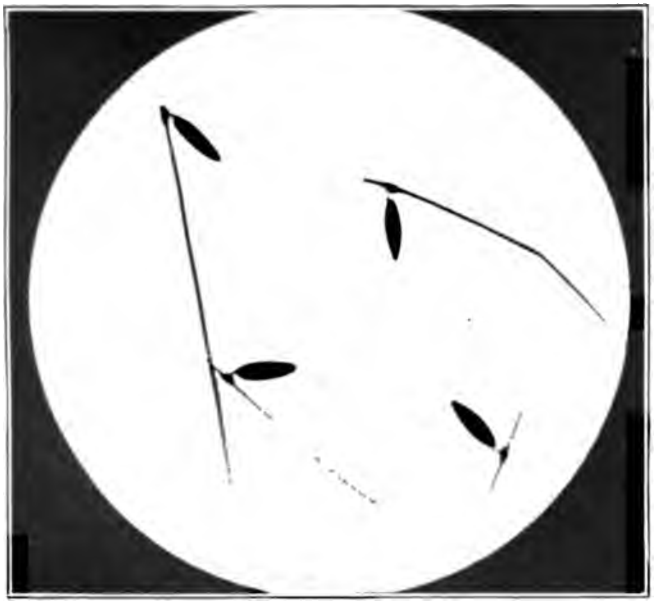


FIG. I.—Warble-fly eggs attached to hairs. Note attachment near base. Slightly enlarged.

FIG. II.

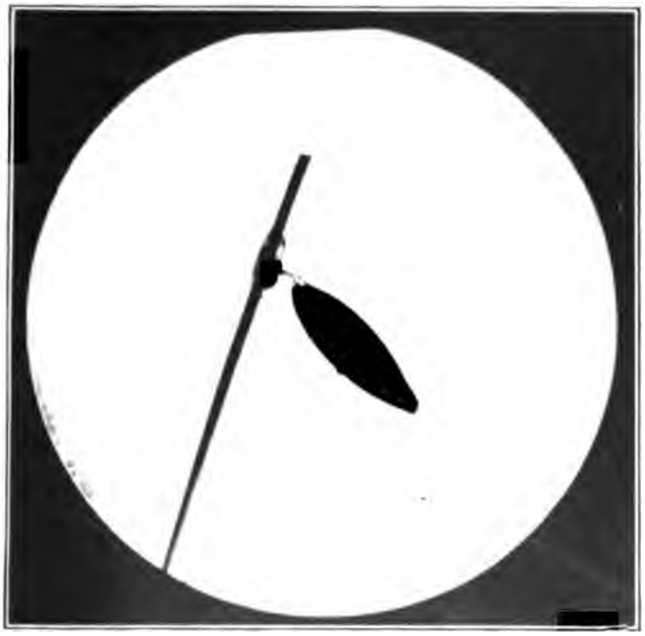


FIG. II.—Warble-fly egg attached to hair. Much enlarged. Photos S. Hadwen

Thus the egg cannot be licked as easily for instance as Gastrophilus eggs. (Horse Bot-fly). Some of the eggs were laid also in places which seem difficult for an animal to reach with its tongue, or at any rate difficult for it to exert the full force. I know that Bot-fly eggs are often laid in inaccessible places, and if I can make an analogy there may be many eggs laid in like places, but I cannot agree with Jost's theory (quoted by Carpenter) who says that the eggs are licked up at once, as soon as laid, which does not seem to me to be tenable; although some may be licked up hair and all. In the first place the eggs are under the hairs, secondly they are well attached to the hairs, and thirdly I found four eggs on a calf's legs

Fig. I

PLATE 5.

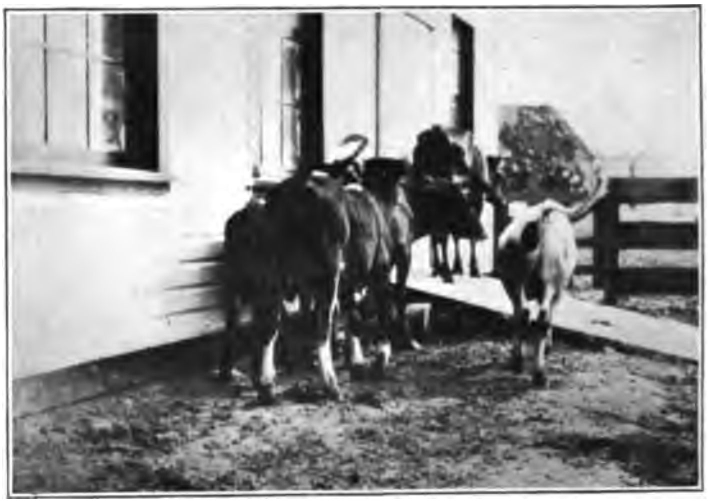


Fig I.—Calves just after the fly had left them.

Fig. II.

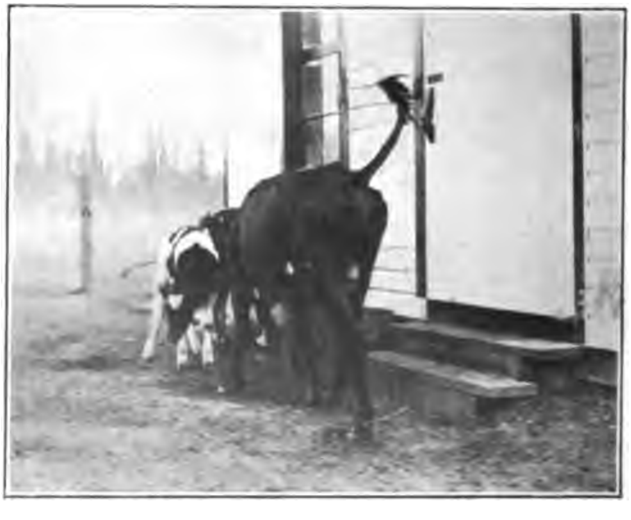


Fig. II.—Calf has just seen fly and is on the point of running away.

FIG. III. PLATE 5.

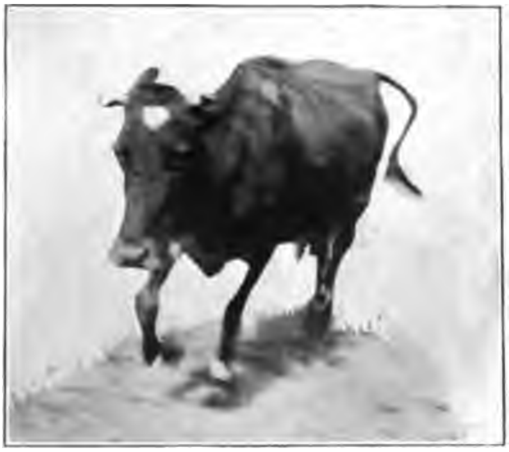


FIG. III.—Cow being chased by fly. Note terrified look of eyes.
Photos S. Hadwen.

which had been at least eighteen days in that position, as the calf had been shut up for that period. The eggs were invariably found singly on the hairs (twenty eggs in all were collected). This does not agree with what Riley says for *H. lineata*, the fly cannot well see where the egg is put, and as it only lays one at a time, it would be difficult for it to find the same place and hair again. This is quite different with the Bot-fly, which lays at the ends of the hairs, and where the egg remains in full view. When a warble fly strikes, it hangs on for a second, evidently holding on with its legs while it fits on an egg.

Egg laying was observed a number of times, and whenever witnessed never occupied more than a few minutes. It may be described as a frenzied sort of process, the fly striking twenty or thirty times rapidly (no accurate account kept) then leaving the animal for fifteen minutes or so, when the process would be repeated. Neumann says that the fly planes over the animal for a time, then darts down, planes again, and so on. I have never observed this; the fly simply attacks the animal with a bull-dog pertinacity as long as it can, unless brushed off, when it may take to another animal. The following notes illustrate this point.

On August 2, at 11.45 a.m., a cow was tied securely to a fence, a warble fly which had been caught a short time before liberated, the net was opened gently, and the fly, after sunning itself for a minute, attacked the cow. It struck several times and was then recaptured. A search was made for eggs in places where the fly was seen to strike, and five eggs were found attached to hairs. At 2.00 p.m., a calf was tied up, the same fly was again liberated; after allowing it to strike a number of times it was caught. Nine eggs were found attached to the hairs in places where it had been seen to strike. The fly was again liberated later, but after striking a few times disappeared.

PLATE 7.

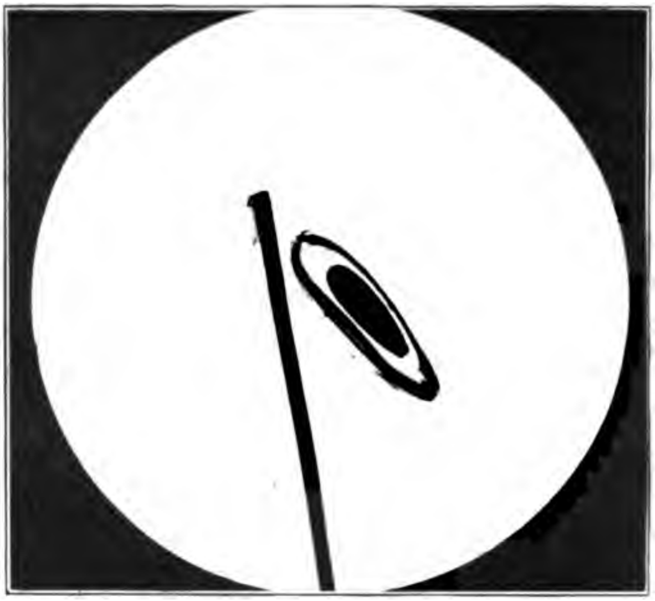


Fig. I.—Larva inside egg much enlarged. Photo S. **Hadwen.**

Larvæ.

Five larvæ were found under the mucous membrane of the œsophagus on August 15, these were secured from a calf which had been tied up inside since July 29. The measurements are as follows:—

3·4 m.m.
4·4 m.m.
4·8 m.m.
3·8 m.m.

The larvæ are similar to those mentioned by Carpenter as being observed by continental writers. They are, however, quite different from the second stage, described by several writers, in having rows of minute spines on the segments, but, as Carpenter has observed, the spines are small and might readily be overlooked. Four larvæ were secured from the œsophagus of one of the experimental cows on November 14. The last time Warble flies were seen near this cow was on August 2, so that the larvæ would be in the neighbourhood of four months old, certainly not more than five. The first time the cattle were chased by flies was on June 8. The measurements of the larvæ are as follows:—

11·5 m.m.
9·3 m.m.
8·8 m.m.
10·3 m.m.

The immature larvæ recorded are presumably those of *H. bovis*, as no specimens of *H. lineata* were captured, nor were any mature larvæ of this species found at Agassiz.

METHOD OF LARVÆ REACHING ŒSOPHAGUS.

With regard to this problem nothing definite has been ascertained, but I believe that a continuation of the study of the egg-laying habits of the fly, and of the eggs 'in situ' on the animal, will yield results. The other stages of the larva have been so fully described by many authors,* that I have nothing to add, except some remarks to make about the mature larva.

PLATE 8.

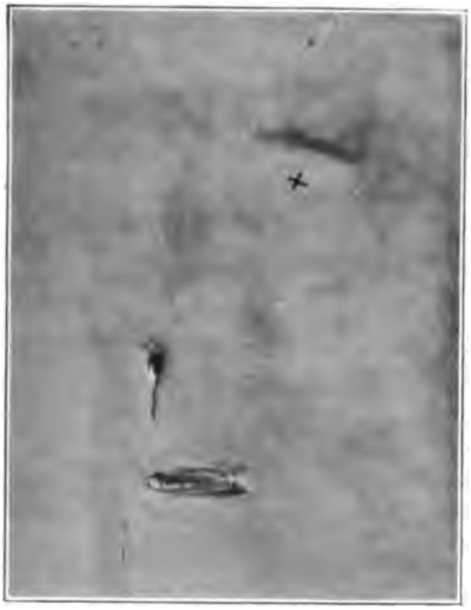


FIG. I.—Larvæ in œsophagus. Mucous membrane incised to show larva. The shadowy outline of another larva may be seen at the point marked +

A number of larvæ were secured from the animals. The method followed was to glue a piece of gauze over a warble from which the larva looked about ready to emerge. Some powdered aloes was mixed with the glue, and a little dusted over the patch. The aloes prevented the cattle to a certain extent from licking the patches, and as they were not put on until the grubs were about ready to emerge, there was generally no very long wait. The emerged larvæ were usually found in the morning; this peculiarity has already been noted by Miss Ormerod and others. The fact is of some importance for stabled animals. Many of the grubs are no doubt carried out in the manure, where a number must perish; this would not apply to such an extent in old, badly kept stables, where there would be an opportunity for the grubs to crawl into cracks and crevices. The first larva was seen to emerge on April 10, the last were found ready to come out on July 2. (One out of four native cows and two out of eighteen cows imported from Ontario the previous autumn). Thus the season in Eastern and Western Canada seems to be about identical. It is reasonable to suppose that the period the larva spends within the body must equal the world over, seeing that an animal's temperature does not vary in different countries. The time of emergence of the larva is however bound to vary owing to climate, as egg-laying coincides with warm weather. It has been said that squeezed out larvæ do not hatch, but one out of three squeezed out larvæ gave rise to a normal puparium, and a male emerged in thirty days. However, these three larvæ were, as far as one could judge, quite ready to come out; they were squeezed out for fear they would be lost.

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*See references, page 20.

The part of the animal in which the larvæ elect to go through their last stages is in the back. It is easy to see the reason for this, they are comparatively safe from injury themselves, and the animal receives a minimum amount of injury also, which is characteristic of nearly all parasites.

FIG. I. PLATE 9.

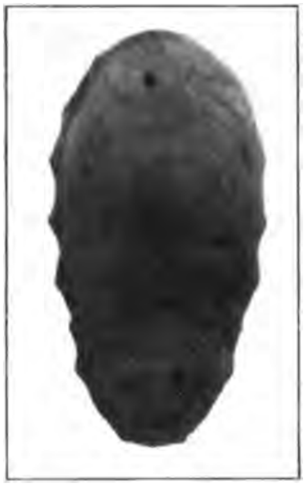

FIG. I.—Puparium enlarged.

FIG. II.

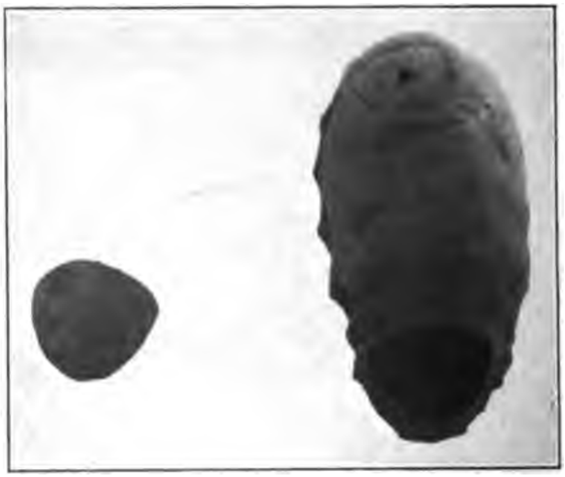

FIG. II.—Puparium with cap attached showing method of emergence of fly.
Photo S. Hadwen.

REMEDIES.

Many remedies are recommended for killing or extracting the grubs. As for the practice of killing the larvæ under the skin by injecting petroleum or applying mixtures to the back, I consider it unscientific, for when the larva dies, its body has to be absorbed. This is likely to take some time, and do the animal harm. The best method, undoubtedly is to squeeze out the warble as early as possible, softening the skin first, in this way the wound will heal up rapidly. Moussu says that in Denmark the various agricultural societies engage men to go round and squeeze out the warbles early in the year; using a small knife to enlarge the opening when necessary. I do not know how tanners would view this, but should imagine the slight extra injury to the hide would be small, and that the resulting scar tissue would be less than is the case when the larvæ are left to come out by themselves. Of course in a small sea-girt country like Denmark, it is theoretically possible to eradicate the fly in this way, but I am afraid that in Canada, under present conditions, it would be an impossibility. Mixtures applied to prevent the fly laying, are, according to all authorities, useless, and many of them are said to be injurious to the hides, and to the animals themselves.

In this country cattle are the favourite hosts of warbles, the only other animals I have seen affected are horses, but rarely so. Railliet records them also for for sheep and man, but remarks that they do not seem to be found in any special part of the body, but wander about and do not reach maturity.

In the Southern States where dipping of cattle is extensively practised for the eradication of ticks, it has been found to result in preventing grubby hides, the dip evidently destroying the eggs or the newly hatched larvæ.

SPECIES OF WARBLES FOUND.

All the full grown warble maggots collected at Agassiz, proved to be those of *H. bovis*. Some of these came from cattle which originated in Eastern Ontario, and arrived here last December, so that this species should be found there also. Two larvæ collected at Mount Lehman, B.C., proved to be those of *H. lineata*. The two species are easy to differentiate by the aid of the spiny armature. In *H. bovis*, the segment in front of the tail (at which end are found the spiracles) is unarmed. In *H. lineata* the last segment is fully armed. There are other differences between the two species, which can be found in the various descriptions.

SUMMARY.

The annual loss to the hides through warbles in Canada is between 25 and 30%. The percentage is based on the following:—

Percentage of grubby hides in warble season 56·55% (January to
July, 6 months. This figure makes an average of 28·27%
for the 12 months.
The annual percentage of grubby hides is 34·22%..34·22% } =27·4%
The percentage of annual damage to hides is..... 19·62%

The warble-fly described here, for the first time in Canada, is *Hypoderma bovis* (De Geer).

The egg is laid close to the skin at the base of the hair, and is cemented on by the ovipositor.

The eggs are laid on the legs.

These facts are of some importance, as for a remedy, some solvent material may be found for the cement, or as a repellant for the fly.

The terror which the fly inspires in cattle is probably due to the insect's persistence, and indifference to the kicks and stamps of the animal.

The first larvæ were found in the œsophagus on August 15, they were under 5 m.m. in length, and were provided with minute spines on all segments.

It is recommended that cattle be housed during the heat of the day, and that the grubs be squeezed out early in the season.

ACKNOWLEDGMENTS.

I am indebted first to Dr. F. Torrance, my chief, for his help and encouragement in the work; to those who so kindly responded to the inquiries concerning the damage done to hides by warbles; to Professor Carpenter, and Dr. L. O. Howard, for specimens; to Mr. P. H. Moore, Superintendent of the Experimental Farm, for his friendly help and assistance during the past summer, and to Dr. C. Gordon Hewitt for reading over this paper and offering suggestions which I have adopted in places, rendering my meaning clearer.

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MACROCENTRUS ANCYLIVORUS ROH. AN IMPORTANT PARASITE OF THE ORIENTAL FRUIT MOTH

BY

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(Contribution from Bureau of Entomology)

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MACROCENTRUS ANCYLIVORUS ROH., AN IMPORTANT PARASITE OF THE ORIENTAL FRUIT MOTH[1]

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INTRODUCTION

An investigation of the oriental fruit moth (*Laspeyresia*) *Grapholitha molesta* (Busck), and its parasites, conducted at Riverton, N. J., in 1925 by Peterson and Haeussler (*4*)[3], showed that the very considerable reduction of the infestation by this pest in peach fruit was brought about chiefly by one species of parasite, a braconid of the genus Macrocentrus. Further studies by the writer (*3*), conducted in the same district from 1925 to 1928, showed that a great reduction in the population of the midsummer and late broods of twig-infesting larvae resulted each season from the severe parasitism by Macrocentrus in the larvae of the earlier broods. The average parasitism by this braconid in larvae collected from twigs ranged from 41 to 55 per cent for the four seasons. In 1925 and 1926, collections of larvae which were feeding in twigs during late June and early July showed parasitism by this one species as high as 92 and 84 per cent.

Cross parasitism and cross-breeding experiments by Stearns (*8*) and by the writer showed this parasite to be identical with that reared by Fink (*2*) from the strawberry leaf roller (*Ancylis comptana* Froel.) and described by Rohwer (*7*) as *Macrocentrus ancylivora*. The experiments consisted in the securing of parasitism of each host species by parasites reared from the other and the mating of female parasites from one host with males from the other, with subsequent propagation on both host species.

Because of the importance of this species as a parasite of the oriental fruit moth, it seemed advisable to make a detailed study of the species in its relation to this host. Its biology as a parasite of the strawberry leaf roller has been reported by Fink (*2*), and Stearns (*8*) published on its biology with reference to both *Grapholitha molesta* and *Ancylis comptana* as hosts. The present study deals entirely with the development of this parasite when bred from the oriental fruit moth.

Preliminary investigations on methods of handling the parasite under insectary conditions and a study of the habits of the species were begun in 1926. The more detailed study was taken up at Riverton in 1927, the insectary studies being started with the first adult parasites that emerged from the earliest parasitized fruit-moth larvae collected in the orchards that spring. In November, 1927, the insectary was moved from Riverton to Moorestown, N. J., a

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[2] The writer expresses his indebtedness to Alvah Peterson, formerly of the Bureau of Entomology of the U. S. Department of Agriculture, under whose direction this study was begun, for valuable advice, and to S. A. Rohwer, R. A. Cushman, and A. B. Gahan, of the Bureau of Entomology, for the determination of specimens. This report is based on investigations conducted prior to July, 1929, when the author was assigned to work in France.

[3] Reference is made by number (italic) to Literature Cited, p. 100.

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distance of about 5 miles, and the work was continued at Moorestown until the last hibernating individuals had emerged in the spring of 1929.

METHODS USED IN STUDYING THE PARASITE

The following methods were used in conducting the study of *Macrocentrus ancylivorus* under insectary conditions with *Grapholitha molesta* as a host: Grapholitha larvae, originating from eggs deposited upon pear leaves in the insectary, were allowed to begin their development by feeding in apples. When the larvae were from 4 to 8 mm long they were removed from the fruit as needed and placed on young, succulent peach twigs. The stems of the twigs were first inserted in a small bottle of water and held in a tight bunch by means of a cotton plug in the neck of the bottle. (Fig. 1.) A bunch of 8 or 10 twigs was found to accommodate from 10 to 15 larvae satisfactorily. Within an hour or two the larvae would spin a light silken web about their bodies to hold themselves against the twigs while eating their way in. The larvae were then in suitable position for being parasitized, and the bottle of twigs was placed in a cage containing parasites of both sexes. Late in the season, when young, succulent peach twigs were no longer available, good results were obtained by placing the partly grown larvae on cross-section slices of apple, about one-eighth inch thick. The larvae soon ate their way into the soft tissue where, owing to the thinness of the slices, the ovipositors of the female parasites could easily reach them when the slices were suspended on wire hooks in the parasite cages.

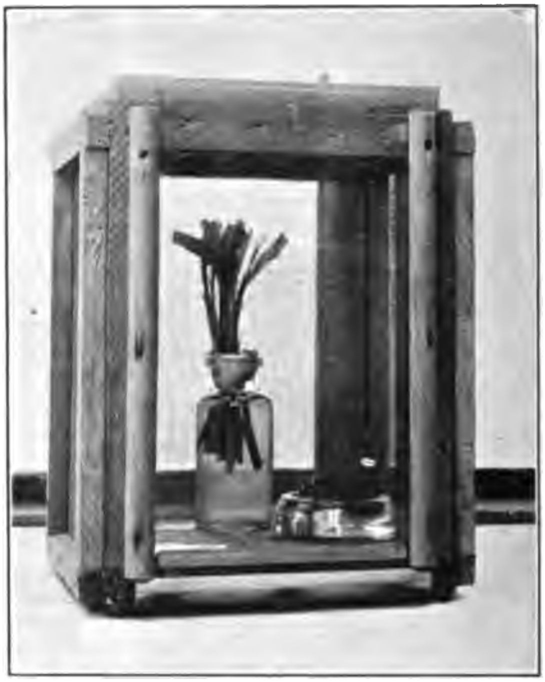

FIGURE 1.—Apparatus used for obtaining parasitism of larvae of *Grapholitha molesta*

The type of cage which proved most satisfactory for obtaining parasitism under insectary conditions is shown in Figure 1. This cage was 6 inches square and 8 inches high, with a solid wooden base and wooden framework covered on three sides and the top with 24-mesh galvanized wire screen. The entire fourth side was a sliding door of heavy transparent celluloid which could be raised for inserting or removing the bottle of twigs or the parasites. A glass caster cup containing a piece of sponge saturated with water was placed in the cage to provide moisture for the parasites. On extremely hot dry days wet cheesecloth, draped over the top and the side of the cage opposite the door, aided materially in prolonging the life of the adult parasites. A high percentage of parasitism resulted when the twigs on which larvae were feeding remained in this cage from 4 to 24 hours, depending upon the number of female parasites present and the prevailing temperature. As a rule, the larvae were kept in the cage for 24 hours.

After the bottle of twigs was taken out of the cage, the twigs were removed and the lower parts of the stems which had been submerged in the water were cut off. The portions of twigs containing the larvae were placed in a jelly glass with one or more apples which provided additional food for the larvae when the twigs had become too dry. A narrow strip of corrugated paper was also placed in the glass, which was then covered with cheesecloth held tightly in place by rubber bands. (Fig. 2.)

When the larvae finished feeding and became full grown they left the fruit and spun their cocoons, usually in the corrugated paper. The glasses were examined once daily, and each corrugation containing a cocoon was cut out, given a record number, and placed in a small homeopathic vial until the adult emerged. Any full-grown larvae found crawling about the glass at the time of examination were placed in individual 2-dram vials stoppered with cloth-covered cotton plugs. These larvae spun their cocoons against the glass, and observations could readily be made of subsequent development without the necessity of disturbing them in any way. Each of these vials was examined once daily, and records were made for every individual whenever any important change occurred. Since it is usually only a matter of a few hours from the time the parasite larva emerges from the body of its host until it spins a cocoon of its own, the emergence of the parasite larva from its host was missed in many instances in the daily observations. Consequently, some of the vials were examined several times a day in order to determine the length of time that the larvae of Macrocentrus feed externally on the host. If a fruit-moth larva pupated it was known to be unparasitized, and subsequent records for that individual pertained to normal fruit-moth development.

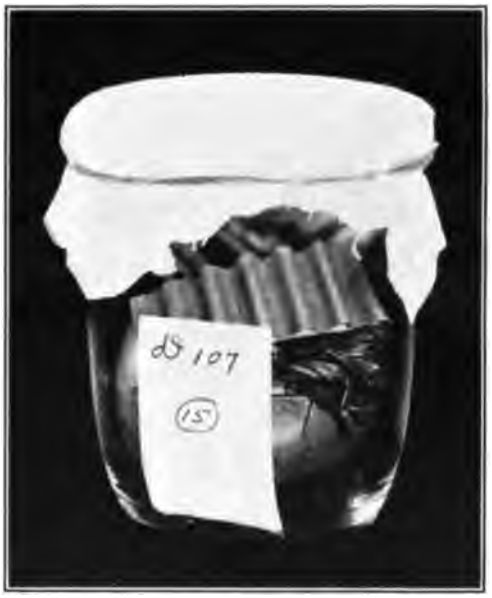

FIGURE 2.—Jelly glass in which parasitized larvae continued feeding

For each individual reared to maturity in observation vials, the dates of moth egg deposition, parasite egg deposition, moth cocoon formation, parasite cocoon formation, pupation of unparasitized fruit moths, and adult emergence of either parasite or moth were recorded. Thus it was possible to compute the length of the life cycle as well as the length of several of the developmental periods for each individual that emerged, whether Macrocentrus or Grapholitha.

THE ADULT

DESCRIPTION

The following description of the adult of *Macrocentrus ancylivorus* (fig. 3) is largely a condensation of the original description by Rohwer (*7*) and subsequent descriptions by Fink (*2*) and Stearns (*8*).

The female averages about 4.5 mm in length, the ovipositor 5.5 mm, antenna 7.5 mm. and wing expanse about 9 mm, male often slightly smaller; General body color pale ferruginous; eyes and ocelli black; antennae uniform dark brown distad of the pedicel, segmentation not clearly distinct except with a strong lens; base and apex of abdomen of both sexes sometimes marked with dark brown; face, ventral part of antennal scape and pedicel, ventral part of thorax, and sometimes venter of abdomen lighter colored, almost yellow; legs yellow.

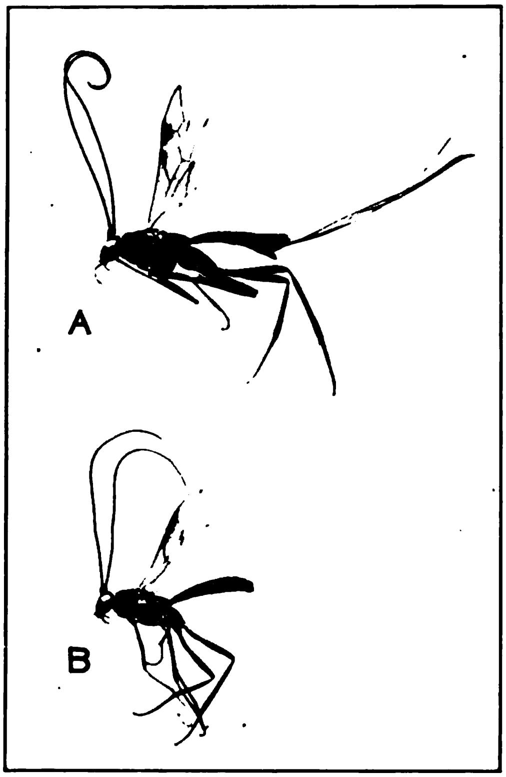

FIGURE 3.—Adults of *Macrocentrus ancylivorus*: A, Female; B, male. × 4.5

SIMILARITY TO MACROCENTRUS DELICATUS CRESS.

In 1926, a few lighter colored adults of Macrocentrus were noted among those emerging from field-collected material. At first these were considered as merely color variations of the one species, but all such individuals were noted in the records, and the specimens were preserved. Similar adults were obtained in 1927, and, since these emerged each season from larvae that had been collected over a period of about two weeks during late June and early July, it was suspected that they might be of a separate species. Mating was never observed between light-colored individuals of one sex and dark-colored individuals of the opposite sex, whereas both light and dark individuals mated readily with similar adults of the opposite sex. It was further noted in 1927 that dark-colored females which had been fertilized always produced dark-colored progeny and that fertile light-colored females always produced light-colored progeny. R. A. Cushman, of the taxonomic unit of the Bureau of Entomology, later determined the light-colored specimens to be a distinct species, *M. delicatus* Cress.

Morphological differences occurring in the two species, as well as characters useful in distinguishing adults of *ancylivorus* from those of *delicatus*, have been very fully discussed by Driggers and Pepper (*1*). The writer found the difference in appearance of the antennae and the difference in general body color to be the most useful characters for distinguishing living adults of one species from those of the other species. That the antennae of *delicatus* are longer and have a segmented or "beaded" appearance whereas those of *ancylivorus* are shorter and are "hairlike" in appearance, as mentioned by the authors cited, was noted by the writer in 1927 and used at that time as a distinguishing character.

MATING

When adults of both sexes of *Macrocentrus ancylivorus* are confined together the male usually attempts mating almost immediately. The wings of the male are raised almost perpendicularly and flutter rapidly as he persistently follows the female. Upon approaching close to the female from the rear the male curls the tip of the abdomen forward from beneath in an endeavor to reach the female. The latter often flies out of reach, and it is sometimes necessary for the male to make several attempts before mating is accomplished.

For 18 observed matings the minimum, average, and maximum duration of the act was 12, 22.5, and 45 seconds, respectively. After mating, the female usually repels further attempts on the part of the same or other males, although in one instance when one female was confined in a 6 by 8 inch glass jar with three males the female was observed mating four distinct times for 30, 12, 19, and 25 seconds, with intervals of 4, 1½, and 11 minutes, respectively. The last three matings were with the same male. A male may mate with several females; thus when more females than males are placed in a cage most of the females usually become fertilized. Mating activity is most noticeable in the cages on warm summer days and during the warmer hours of the day in spring and fall.

PROPORTION OF SEXES PRODUCED

More females than males of *Macrocentus ancylivorus* are recovered from larvae of the oriental fruit moth parasitized normally in the orchards in the vicinity of Riverton, N. J. For the three consecutive seasons, 1926, 1927, and 1928, the percentage of female parasites which emerged from parasitized larvae collected in infested peach twigs was consistently greater than the percentage of males. During the respective seasons, 55.2, 55.5, and 53.4 per cent of the parasites produced were females. A total of 2,642 adult parasites were reared from fruit-moth larvae parasitized in the field over the 3-year period. Of these, 1,443, or 54.6 per cent, were females and 1,199, or 45.4 per cent, were males.

A greater proportion of female parasites was also obtained from larvae of the strawberry leaf roller parasitized in the field in the same region. Collections of larvae of this host species in 1927 and 1928 produced 58.1 and 58.8 per cent female parasites during the respective seasons. From the total of 219 parasites that emerged during the two years, 128, or 58.4 per cent, were females and 91, or 41.6 per cent, were males.

A consistently greater proportion of females, such as occurs under natural conditions, is not always produced when this species is bred under insectary conditions. Of the total number of parasites reared from Grapholitha in the insectary in 1927 in connection with the life-history studies, 64.4 per cent were females. In similar rearings in 1928 only 48.8 per cent were females. However, from the total of 1,562 parasites bred from this host under insectary conditions during the two seasons 894, or 57.2 per cent, were females and 668, or 42.8 per cent, were males.

For securing parasitism of larvae in the insectary, parasites of both sexes were always placed in the oviposition cages. However, it is quite possible that in some instances parasitism occurred before all of the female parasites had been fertilized. Very probably this happened on several occasions in 1928 when, owing to a shortage of males, more female than male parasites were placed in the oviposition cages. This may explain, in part at least, the high percentage of male parasites that was produced during that season, since unfertilized eggs of *Macrocentrus ancylivorus* yield males only.

LONGEVITY

Twenty-two separate experiments were conducted during the season of 1927 to determine the length of adult life when parasites of both sexes were confined together in cages such as those previously described, with either fruit-moth larvae or larvae of the strawberry leaf roller. Records were obtained from a total of 115 female and 79 male parasites. The minimum, average, and maximum length of life for both sexes confined together was 1 day, 6.7 days, and 14 days, respectively. The highest average longevity for the females in any single experiment was 11.1 days, for the males 11.3 days, and for the two sexes together 10.6 days.

For eight unfertilized females that were confined in a cage without males, but provided with Grapholitha larvae in which to oviposit, and fed a 10 per cent solution of honey water, the minimum, average, and maximum length of life was 13, 17.9, and 29 days. When freshly emerged males were confined in a cage with no females and no larvae present, but fed the 10 per cent solution of honey water, the respective duration of life was 7, 10.3, and 19 days.

OVIPOSITION

Oviposition by *Macrocentrus ancylivorus* on *Grapholitha molesta* is most readily accomplished when the larva of the host species is in a position from which it can not easily escape the thrusts of the ovipositor of the female parasite. The most favorable position for attack seems to be when the larva is feeding within a peach twig or a thin slice of apple, or is inclosed in a light feeding web preparatory to entering a twig. Successful oviposition has never been observed by the writer when the host larva was not in some such fixed position. If free to escape, the larva wriggles out of reach at the first touch of the ovipositor and thus frightens the parasite.

When a number of full-grown larvae of the oriental fruit moth were killed in hot water, inserted in nail punctures in a piece of dried cornstalk, and placed in an oviposition cage, female parasites oviposited readily in the bodies of the dead larvae. The dead hosts dried up, however, before the parasite eggs could develop. On several occasions females were observed endeavoring to parasitize full-grown Grapholitha larvae that were wandering about the cage or other container, but the larvae always managed to escape the thrust of the ovipositor, and in no instance was such attempted parasitization successful.

Females of this species have been observed ovipositing at practically all hours of the day during warm summer weather. On several occasions, when a flash light was suddenly turned on the oviposition cages after dark, parasites were observed ovipositing as late as 10 p. m.

Under insectary conditions a female parasite will oviposit several times in the same larva. One female was observed to puncture a larva 17 times and another female oviposited 13 times in a single larva. Dissections of 12 hibernating Grapholitha larvae which had been parasitized in cages in the insectary showed that, although superparasitism occurs, only one parasite larva survives regardless of the number of eggs deposited. Each host larva contained one living parasite larva. In 11 of the host larvae from 1 to 58, or a total of 131, head capsules and bodies of dead parasite larvae were also present. The remaining host larva contained no sign of superparasitism.

PARTHENOGENESIS

Unfertilized females can deposit eggs that will mature, but the progeny are always males. In 1927 a cage containing 10 freshly emerged unfertilized females was started on July 28. From numerous insectary-bred larvae of the oriental fruit moth which were placed in this cage a few at a time, a total of 63 parasites was produced, all of them males. When some of these males were allowed to mate with unfertilized females emerging from larvae of the same host that had been parasitized in the field, normal progeny of both sexes were produced.

STAGE OF HOST ATTACKED

Macrocentrus ancylivorus will attack the feeding larvae of the oriental fruit moth in any stage of development, from the very small first-stage larvae to those that are nearly full grown. Table 1 shows the parasitism by this species which occurred in Grapholitha larvae of various sizes taken in weekly collections of infested twigs from orchards near Riverton during the season of 1927. Each larva was removed from the twig within an hour after it had been collected, and its length in millimeters was recorded. The larvae were divided into three groups, according to length, and placed on apples in jelly glasses to continue their development. One group contained larvae measuring from 1.5 mm (recently hatched) to 3.5 mm, inclusive, another group contained those measuring over 3.5 to 6.5 mm, inclusive, and the third group contained those measuring over 6.5 mm. Practically all the larvae of the third group were in the last larval instar. The table shows, for each respective size group, the number of Grapholitha larvae collected, the total number of individuals that completed development and emerged as adults (including moths and all species of parasites), and the number of adult *M. ancylivorus* that emerged. The percentage of all individuals that reached maturity, based on the number of larvae collected, and the percentage of *M. ancylivorus* that emerged, based on the total emergence, are also given for each of the three groups and for the combined total. It will be noted that the percentage of individuals reaching maturity increased decidedly with each increase in the size of the larvae when collected. This is explained, in part at least, by the fact that more of the smaller larvae were injured in handling and failed to enter the new food medium after having been removed from the twigs. There was also an increase in the percentage of Macrocentrus that emerged from the larvae of each progressive size.

TABLE 1.—*Numbers and percentages of individuals reared to maturity and Macrocentrus ancylivorus adults emerged from Grapholitha molesta larvae of various sizes taken in field collections of infested peach twigs at Riverton, N. J., 1927*

Grapholitha larvae		Adults, both moths and all parasites, emerging		Macrocentrus ancylivorus emerging	
Size when collected	Collected				
	Number	*Number*	*Per cent*	*Number*	*Per cent* [a]
1.5 to 3.5 mm	900	193	21.44	97	50.26
Over 3.5 to 6.5 mm	974	361	37.06	193	53.46
Over 6.5 mm	1,015	651	64.14	379	58.22
Total or average	2,889	1,205	41.71	669	55.52

[a] Based on total emergence of moths and parasites.

Under insectary conditions it was also found that the percentage of individuals reaching maturity was greater with each increase in the size of the larvae at the time these were transferred from apples to twigs before being placed in the parasite oviposition cages. The majority of the larvae used in the life-history studies ranged in length from 4 to 8 mm, inclusive. During both 1927 and 1928, fruit-moth larvae ranging from 4 to 6.5 mm in length were more heavily parasitized than those measuring 7 mm and over. The percentages of parasites that emerged from a considerable number of larvae that had been separated into these two size groups were 56.98 and 47.96, respectively, in 1927, and 83.33 and 70.81, respectively, in 1928. There were not sufficient numbers of larvae measuring from 1 to 3.5 mm used in the insectary studies to provide comparative data on the percentage of parasitism occurring in these smaller larvae. Of the total number of adults reared from all larvae subjected to the attack of Macrocentrus in the insectary in 1927, 1928, and in both seasons combined, 54.72, 71.10, and 61.23 per cent, respectively, were parasites.

Parasitism of *Grapholitha molesta* in the field is confined largely to those larvae of the first, second, and a portion of the third broods which feed within the peach twigs, for when larvae of the late broods enter the fruit it is difficult for the female Macrocentrus to reach them with her ovipositor. In 1925 a large number of larvae of the oriental fruit moth were removed from Elberta peaches at harvest time. From these larvae, 627 moths and only 1 *M. ancylivorus* emerged. The parasitism by this species in larvae that had infested the twigs in that same orchard earlier in the season was very high.

This parasite does not attempt to attack full-grown Grapholitha larvae that have formed cocoons, nor does it attempt to oviposit in naked Grapholitha pupae.

REPRODUCTIVE CAPACITY

Four females of *Macrocentrus ancylivorus* were mated within a few hours after they had emerged and were then placed in cages, only one parasite to a cage, and each was provided with 50 larvae of the oriental fruit moth feeding on bundles of twigs. A new lot of 50 larvae was placed in each cage every second day until the parasite died. The four females lived 8, 4, 5, and 6 days; and the number of progeny produced by each individual was 40, 28, 29, and 22, respectively, 119 in all, or an average of 29.75 each.

LIFE HISTORY AND COMPARATIVE RATE OF DEVELOPMENT OF THE PARASITE AND THE HOST

The writer had planned to make a detailed study during the season of 1929 of that period in the development of *Macrocentrus ancylivorus* during which the parasite lives within the body of the host larva. Circumstances, however, prevented continuation of investigations with this parasite after the spring of 1929. Consequently, information is not available regarding the duration of the egg stage and the number and duration of the larval instars. Data concerning the minimum, average, and maximum number of days required for the several periods of development and for the complete life cycle of *M. ancylivorus* and its host *Grapholitha molesta* during 1927 and 1928 and for the two seasons combined are presented in Table 2. These data are recorded separately for transforming[4] and for wintering[4] individuals. In each instance the number of parasites or fruit moths from which observations were made is indicated.

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[4] The terms "transforming" and "wintering" are used here in the same sense in which they were used by Peterson and Haeussler (*6*) in their life-history study of the host species. "Transforming" refers to those individuals which, completing their life cycle, transform to adults the same season the eggs are deposited; "wintering" refers to those individuals which hibernate and do not become adults until the following season.

TABLE 2.—*Average, minimum, and maximum number of days required for several periods of development and for the life cycle of Macrocentrus ancylivorus and Grapholitha molesta in 1927 and 1928, and the number of individuals on which data are based, Riverton and Moorestown, N. J.*

Period of development	Time required for development										Individuals observed					
	Transforming					Wintering					Transforming			Wintering		
	1927	1928	Two years combined			1927	1928	Two years combined			1927	1928	Two years combined	1927	1928	Two years combined
	Average	Average	Average	Minimum	Maximum	Average	Average	Average	Minimum	Maximum						
MACROCENTRUS ANCYLIVORUS																
	Days	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Days*	*Number*	*Number*	*Number*	*Number*	*Number*	*Number*
Parasite egg deposition to formation of host cocoon	8.3	10.5	9.4	2	31	12.2	25.9	16.7	7	44	287	313	600	33	16	49
Host cocoon formation to emergence of parasite larva	7.8	6.0	6.9	1	23	200.5	169.2	190.31	141	212	122	126	248	33	16	49
Emergence of parasite larva to formation of its cocoon	.6	.5	.5	[a].5	4	2.4	1.4	2.1	[a]0.5–1	6	122	126	248	33	16	49
Formation of cocoon to emergence of adult	16.4	14.0	15.2	9	69	37.6	40.5	38.5	30	52	287	313	600	33	16	49
Life cycle [b] (observation vials only)	33.1	31.1	32.1	21	91	252.7	237.1	247.6	224	262	287	313	600	33	16	49
Life cycle (including cocoons in corrugated paper)	32.9	29.7	31.2	20	91	243.4	243.1	243.3	216	280	582	653	1,235	259	68	327
GRAPHOLITHA MOLESTA																
Egg deposition to formation of cocoon	22.0	28.2	24.6	14	51	31.8	42.4	35	13	89	139	99	238	66	28	94
Formation of cocoon to pupation	3.9	3.9	3.9	1	13	210.6	184.4	202.8	108	256	139	99	238	66	28	94
Pupation to emergence of adult	11.6	9.3	10.6	7	15	31	34.3	32	9	40	139	99	238	66	28	94
Life cycle (observation vials only)	37.5	41.4	39.2	26	61	273.3	261.1	269.7	236	308	139	99	238	66	28	94
Life cycle (including cocoons in corrugated paper)	35.7	37.9	36.3	23	62	275.2	272.6	274.3	236	337	479	173	652	217	120	337

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[a] Approximation.

[b] "Life cycle" includes the complete development, from deposition of the egg to emergence of the adult.

DEVELOPMENT OF THE PARASITE LARVA WITHIN THE HOST

The parasite larva which hatches from the egg deposited within the body of the partly grown Grapholitha larva spends the greater portion of its larval life as an internal parasite. When the Grapholitha larva has finished feeding in its last stage and reached the quiescent period within its cocoon the parasite larva, having attained its last instar, makes its way out of the host. During the two seasons of 1927 and 1928 (combined), unparasitized transforming Grapholitha required from 14 to 51 days, or an average of 24.6 days, from the time the egg was deposited until the cocoon was spun; and unparasitized wintering Grapholitha required from 13 to 89 days, or an average of 35 days. Parasite eggs which produced parasites transforming during the same season were deposited in the host larvae from 2 to 31 days, or an average of 9.4 days, before the formation of the host cocoon; and those producing wintering parasites were deposited from 7 to 44 days, or an average of 16.7 days, before the formation of the Grapholitha cocoon.

Transforming fruit-moth larvae, when unparasitized, pupate in from 1 to 13 days, or an average of 3.9 days after the cocoon has been spun. The transforming parasite larva emerges from the body of the Grapholitha larva in from 1 to 23 days, or an average of 6.9 days, after the host larva has constructed its cocoon. Therefore, the transforming parasite larva emerges from the body of the host on an average of 3 days after the fruit-moth larva would normally have pupated.

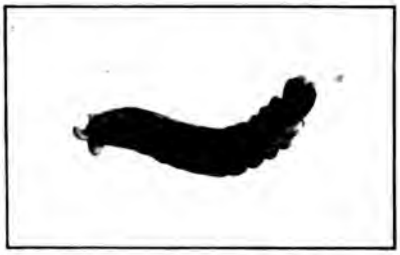

FIGURE 4.—Hibernating larva of *Macrocentrus ancylivorus*. × 12.5

Wintering parasite larvae remain within the body of the hibernating host larvae throughout the winter. The hibernating larva of *Macrocentrus ancylivorus* (fig. 4) averages 2.63 mm in length and 0.43 mm in width. The heavily sclerotized, light-brown head measures 0.24 mm in length by 0.27 mm in width and bears a pair of very strong, heavily sclerotized mandibles. The body, which is of a dirty yellowish white, consists of 13 segments besides the head and is terminated by three hooklike prolongations of the anal segment. The hibernating larvae coincide in all respects with the description given by Fink (*2*) of the mature first-instar larva of this species.

In the spring, the hibernating larvae attain their last larval instar as internal feeders and then come out of the body of the host, the first parasite larvae appearing externally a few days after the first unparasitized fruit-moth larvae have pupated. Parasite eggs which produce hibernating parasites are deposited later in the fall than are Grapholitha eggs which produce hibernating individuals. The average length of time from formation of the host cocoon in the fall to emergence of the parasite larva the following spring (190.3 days) is considerably shorter than the period from the formation of the cocoon to the transformation to pupa (202.8 days) for unparasitized wintering individuals of the host species. Fruit-moth larvae that are parasitized by *M. ancylivorus* do not pupate, as the host larva is always destroyed previous to pupation.

DESTRUCTION OF THE HOST

When the parasite larva comes out of the host it lies parallel to the body of the host larva with its head embedded in the hole from which it emerged. It then proceeds to feed on its former host until nothing remains but the sclerotized parts and the shriveled skin. During warm summer weather the average transforming parasite larva finishes feeding in from about 12 to 18 hours after emerging from its host. Wintering parasites, the larvae of which emerge from the host during cool spring weather, require an average of about 2 days to complete their feeding. While feeding externally, the dirty yellowish-white parasite larva shows up distinctly against the darker grayish pink of the host larva. (Fig. 5.) It increases rapidly in size as it feeds, and when full grown is from one-half to two-thirds the size of its former host, averaging about 5 to 6 mm in length.

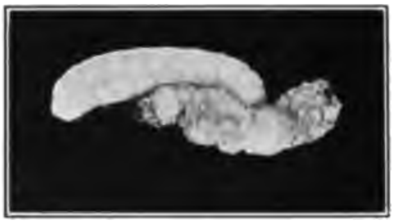

FIGURE 5.—Partly grown Macrocentrus larva feeding on a larva of the oriental fruit moth. × 5

FORMATION OF THE PARASITE COCOON

Shortly after the full-grown Macrocentrus larva has finished feeding, construction of the parasite cocoon begins. This cocoon is constructed within the old cocoon of the former host. The shriveled skin, sclerotized head, and thoracic and anal shields of the host larva also remain within the host cocoon. At first a web of very fine, white, silky threads appears about the parasite larva. (Fig. 6, A.) As construction continues the cocoon gradually becomes light brown in color. The completed cocoon (fig. 6, B), which makes a very strong protection about the prepupa and pupa, is dark amber in color and oval in shape and averages from 5.5 to 6.5 mm in length by 1.5 to 2 mm in width. About 24 hours are required for the spinning of the cocoon. The adult emerges from the cocoon by making a slit which opens the round, somewhat thickened anterior end. (Fig. 7.)

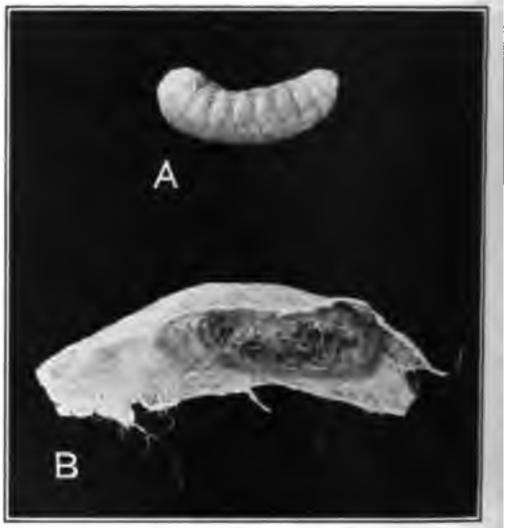

FIGURE 6.—Cocoon of *Macrocentrus ancylivorus*: A, Full-grown larva beginning to construct cocoon; B, completed cocoon shown within a cocoon of the oriental fruit moth. × 4.5

PREPUPAL AND PUPAL PERIODS

The time required from formation of the parasite cocoon to emergence of the adult, including the prepupal and pupal periods, averaged 15.2 days for transforming parasites (Table 2), with a minimum of 9 days and a maximum of 69 days. For wintering parasites, the period of time within the parasite cocoon averaged considerably longer, 38.5 days, with a minimum of 30 days and a maximum of 52 days. This was due to the fact that the cocoons of hibernating parasites were all spun early in the spring when the weather was cool, and thus the prepupal and pupal periods were of longer duration.

The time spent within the cocoon by unparasitized Grapholitha has been separated into two periods, prepupal and pupal. (Table 2.) The former period, indicated as "formation of cocoon to pupation" averaged 3.9 days for transforming moths and 202.8 days for wintering moths. The remainder of the time within the cocoon, "pupation to emergence of adult," required an average of 10.6 days for transforming Grapholitha and 32 days for wintering ones, the minimum and maximum time for this period being, respectively, 7 and 15 days for transforming individuals, and 9 and 40 days for wintering individuals.

LIFE CYCLE OF PARASITE AND HOST

The life cycle of *Macrocentrus ancylivorus*, from deposition of the egg to emergence of the adult, averaged 32.1 days for the 600 transforming parasites and 247.6 days for the 49 wintering parasites in observation vials during the two seasons. Records on the life cycle were also available from parasites the host cocoons of which had been spun in corrugated paper. When the two sets of records were combined, the life cycles of the 1,235 transforming parasites were found to average 31.2 days, the minimum and maximum life cycles being 20 days and 91 days, respectively. The minimum, average, and maximum life cycles of the 327 wintering parasites in the combined records were 216, 243.3, and 280 days, respectively.

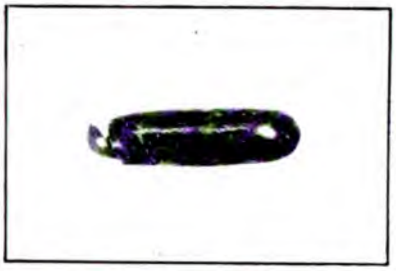
FIGURE 7.—Macrocentrus cocoon from which the adult has emerged, showing opening at the anterior end. × 4.5

For unparasitized fruit moths reared in observation vials during the two seasons the average life cycles were 39.2 days for 238 transforming moths and 269.7 days for 94 wintering moths. When records of those individuals that had spun cocoons in corrugated paper were included, the minimum, average, and maximum life cycles of 652 transforming moths were 23, 36.3, and 62 days, respectively, and the corresponding life cycles of 337 wintering moths were 236, 274.3, and 337 days, respectively.

The life cycle of the parasite is in each instance considerably shorter than that of the host species. The average life cycle of all transforming parasites was 5.1 days shorter than that of all transforming moths, and for all wintering parasites 31 days shorter than for all wintering moths.

AVERAGE LIFE CYCLE AND RELATION TO TEMPERATURE

The average number of days required for the life cycle of transforming parasites and moths maturing from eggs deposited on each day throughout the seasons of 1927 and 1928 is shown in Figures 8 and 9. The average life cycles of all transforming Macrocentrus that emerged as adults throughout the season are indicated by the height of the solid day line above the base line on the date of oviposition. The average life cycles of transforming Grapholitha that emerged as adults throughout the season are similarly shown by the height of the dotted day line above the base line on each oviposition date.

The relation between prevailing temperature and the length of the life cycle in each case is also shown. The height of the solid and dotted temperature lines for each date indicates the average of all temperatures which existed during the average life-cycle periods of all individuals that emerged as adults from eggs deposited on that date, for parasites and moths, respectively. For example, the average life cycle of all transforming Macrocentrus, the eggs of which were deposited on June 27, 1927, was 26 days; and the average temperature recorded for that period, as indicated by the solid temperature line on that date, was 72.8° F. This is the average of all temperatures for 26 days, starting with June 27, based on thermograph readings at 2-hour intervals throughout the entire period. The average life cycle of all transforming Grapholitha, the eggs of which were deposited on that same date, was 32.3 days; and the average temperature recorded for that period, as indicated by the dotted temperature line on June 27, was 73.2° F. This is the average of all temperatures for 32.3 days starting with June 27.

SPRING EMERGENCE OF HIBERNATING PARASITES AND MOTHS

It has previously been pointed out by the writer (*3*) that, in the vicinity of Riverton, N. J., adults of *Macrocentrus ancylivorus* emerge sufficiently early in the spring under field conditions to attack the first fruit-moth larvae which enter twigs.

During the winter of 1927–28 Grapholitha larvae which had been subjected to parasitism by Macrocentrus in the insectary during the fall of 1927 were allowed to hibernate outdoors exposed to natural conditions of temperature and weather. The larvae had constructed cocoons in narrow strips of corrugated straw paper which were firmly tacked in open screen cages, identical with one of the types of cages (fig. 10) employed by Peterson and Haeussler (*5*) in conducting spring-brood emergence studies of the oriental fruit moth. Two cages, each containing approximately 230 cocoons, were hung on a post. One cage was placed on the south side of the post, as close to the ground as possible, and the other was placed on the north side of the post, about 5 feet above the ground. Previous experiments with hibernating oriental fruit moths and codling moths had shown that the larvae in cages placed in these two positions were subjected to the two extremes of temperature and sunlight during the spring to which larvae normally hibernating on the trunks of trees in the orchard are exposed, and that, consequently, the earliest and latest emergence of adults in the spring occurs in the south-low and north-high cages, respectively.

As had been expected, the first emergence of parasites in the spring of 1928 occurred in the south-low cage, and, as a matter of fact, all

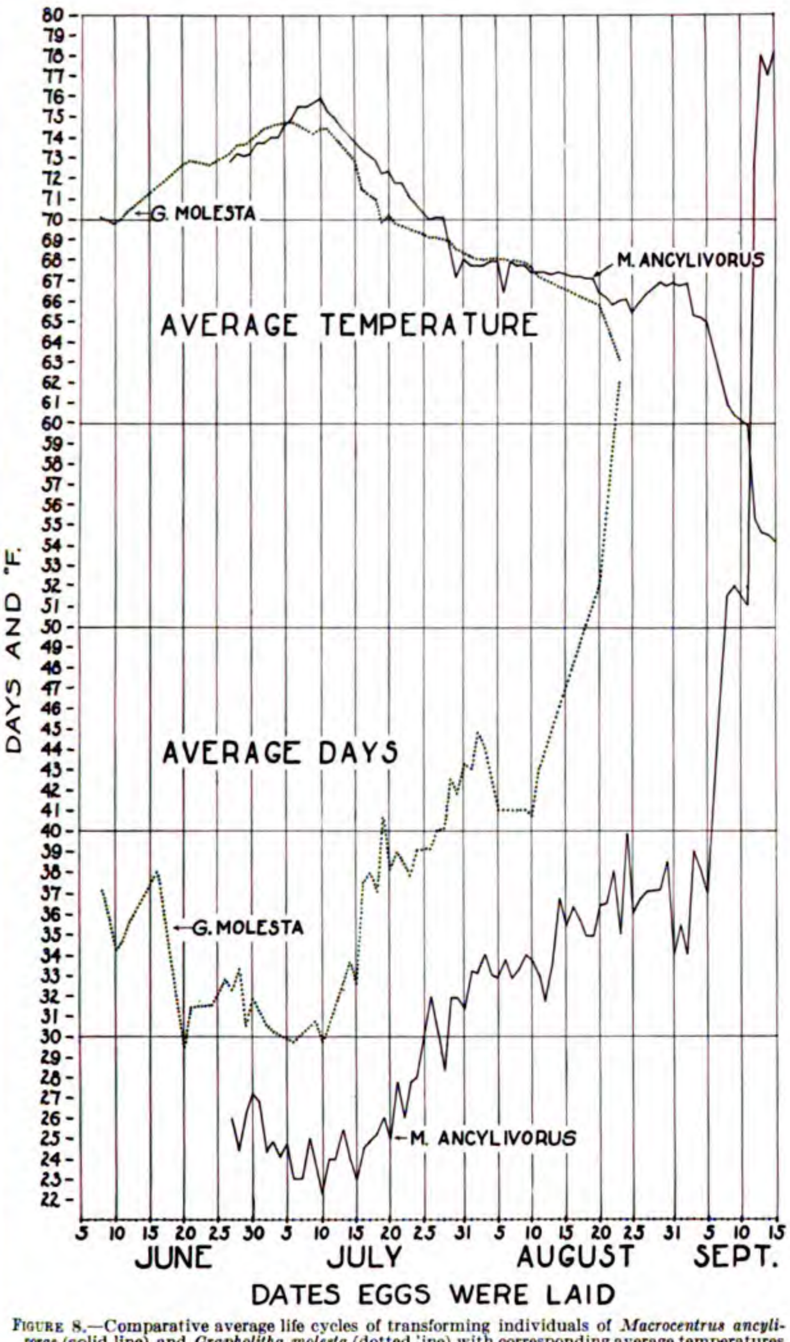

FIGURE 8.—Comparative average life cycles of transforming individuals of *Macrocentrus ancylivorus* (solid line) and *Grapholitha molesta* (dotted line) with corresponding average temperatures in 1927. The life cycle is indicated by the height above the base line (at 0, not shown) on the date on which the eggs were laid. The temperature indicated for that date is the average temperature for the entire life cycle that began on that date

the parasites in this cage had emerged two days before emergence began in the north-high cage. The first adult Macrocentrus appeared in the south-low cage on May 11, the peak of emergence (that is, the date on which 50 per cent had emerged) occurred on May 17, and the last parasite emerged on May 19. In the north-high cage, first emergence was on May 21, the peak of emergence was reached on May 24, and the last emergence occurred on June 12.

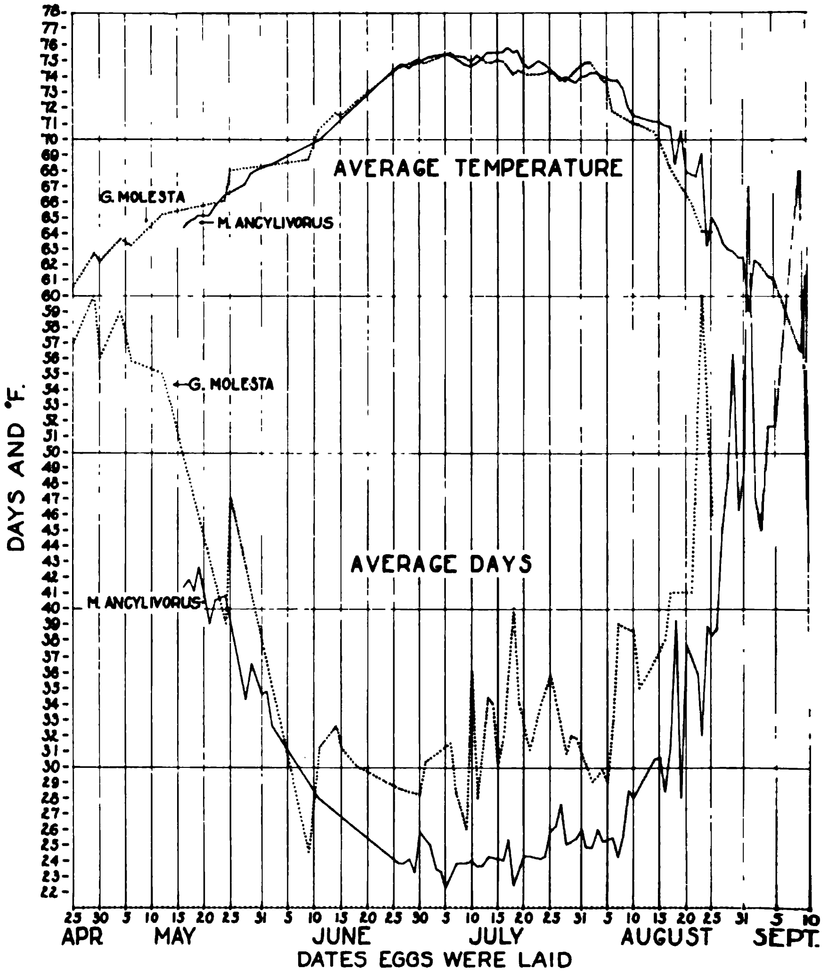

FIGURE 9.—Comparative average life cycles of transforming individuals of *Macrocentrus ancylivorus* (solid line) and *Grapholitha molesta* (dotted line) with corresponding average temperatures in 1928. The life cycles and temperatures are indicated as explained in the legend of Figure 8 and in the text

Two similar cages containing unparasitized larvae of the host species had been placed in corresponding positions on a near-by pole. The first adult moth emerged in the south-low cage on April 8, the peak of emergence in this cage was reached on April 30, and last emergence occurred on May 7. In the north-high cage the first moth emerged on May 8, the peak of emergence occurred on May 16, and the last moth emerged on June 12.

By combining the emergence data from the south-low and north-high cages, in the case of both the parasite and the moth, a general idea is obtained of the spring emergence of the two insects closely approximating that which normally occurs in the peach orchards, assuming that hibernating cocoons under natural conditions are more or less evenly distributed in these two extreme and all intermediate locations.[5]

The comparative emergence of the parasite and the host species in the outdoor cages and in the insectary during the spring of 1928 is shown in Figure 11. The solid lines represent parasite emergence and the broken lines emergence of the moth. The dates of first and last emergence are indicated on the base line and the peak of the triangle denotes the date on which the peak of emergence was reached in each instance.

It will be noted that in the outdoor cages the first moth emerged on April 8, more than a month earlier than the emergence of the first parasite on May 11. Of both species, the earliest individuals to emerge were males, the first female moth emerging on April 20, which was 25 days before the emergence of the first female parasite on May 15. The peak of moth emergence was reached on May 4, which was 19 days before the peak of parasite emergence on May 23. The last individuals of both parasite and host species emerged on June 12.

Spring emergence was considerably delayed in the case of hibernating cocoons which had been kept in the insectary throughout the winter and spring. As shown in Figure 11, the first moth from this material emerged on May 10, the peak of emergence was reached on May 30, and the last moth emerged on July 1. This represents a delay of 32, 26, and 19 days, respectively, as compared to moth

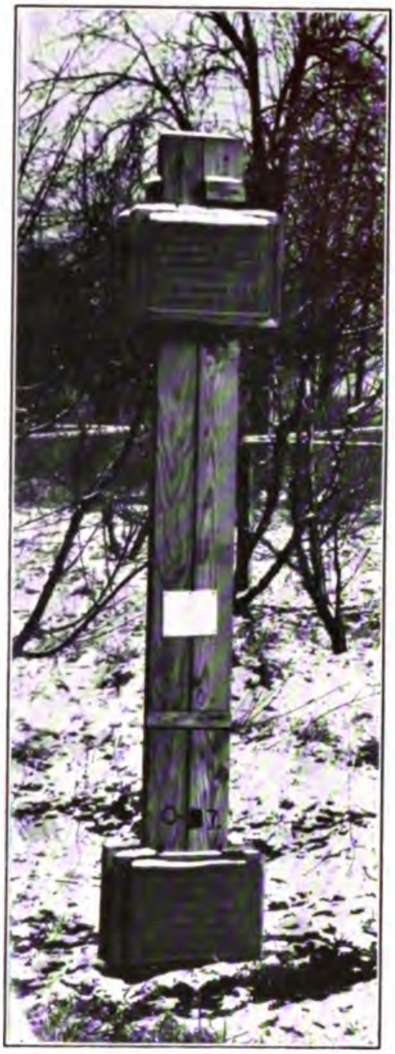

FIGURE 10.—Type of outdoor hibernation cage from which the spring-emergence records were obtained for both parasites (*Macrocentrus ancylivorus*) and moths (*Grapholitha molesta*)

emergence in the outdoor cages. In the case of the parasites the delay was not so great. Nevertheless a retardation of emergence occurred, the first adult (a female) appearing on May 19, 8 days later than the first parasite emerged in the outdoor cages. The peak of emergence was delayed 4 days, being reached on May 27; and the last parasite emerged on June 13, only 1 day after the last individual emerged in the outdoor cages. Thus it is apparent that, in the case of the parasites as well as the moths, a closer approximation to the normal spring emergence can be obtained by the use of outdoor hibernation cages. In the insectary, the first parasite emerged 9 days later than the first moth. The peak of parasite emergence, however, was reached 3 days in advance of the peak of moth emergence, and the last moth emerged 18 days later than the last parasite.

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[5] A closer approximation of normal orchard emergence would undoubtedly have been obtained by the use of eight cages, four adjacent to the ground and four 5 feet above the ground, with one cage of each group facing north, east, south, and west. Data regarding the emergence of the host species were available for the entire eight cages but since sufficient hibernating parasite material was available for only two cages, the emergence data for the moths, as given here, were taken from only the two corresponding cages of hibernating Grapholitha material.

SEASONAL EGG DEPOSITION

A comparison of the periods during which fruit-moth eggs and parasite eggs were deposited in 1927 and 1928, from which transforming and wintering individuals of Grapholitha and Macrocentrus subsequently emerged, is presented in Figure 12. The dates on which the first and last eggs producing transforming and wintering individuals were deposited are indicated in each instance by the range of the horizontal lines, the broken and solid lines representing the fruit-moth eggs and parasite eggs, respectively.

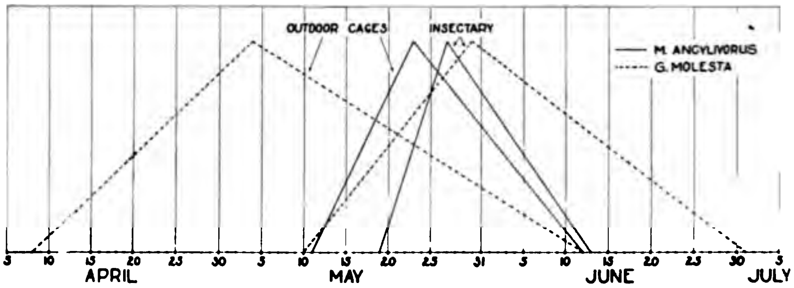

FIGURE 11.—Comparative emergence of adults of *Macrocentrus ancylivorus* and *Grapholitha molesta* during the spring of 1928 from larvae that hibernated in outdoor cages and from larvae that hibernated in the insectary

In 1927, the first Grapholitha eggs of the season were deposited in the insectary on April 23 by females which emerged in outdoor cages, but the first eggs on which developmental records were kept in connection with the parasite studies were deposited on June 8. The last eggs that produced transforming moths were deposited on August 23. The first eggs producing wintering Grapholitha were deposited on July 27. Thus both transforming and wintering fruit moths developed from eggs deposited between July 27 and August 23, inclusive. The last eggs that produced wintering Grapholitha, which were also the last eggs of this species laid that season, were deposited on September 24.

Since it was not possible to begin the parasite life-history studies in 1927 until adults of the first brood began to emerge from parasitized host larvae collected in the field, the exact date on which the first parasite eggs of that season were deposited is not known. However, the first Grapholitha larvae found in peach twigs in the orchard that season, which were found on May 11, were parasitized by *Macrocentrus ancylirorus*. Therefore, it is certain that female parasites had emerged from hibernating material and deposited eggs in the field by that date. The parasite studies in the insectary, which were started with the deposition of the first eggs of the second brood on June 27, showed that the last parasite eggs that produced transforming parasites were deposited on September 15. The first eggs that produced wintering parasites were deposited on August 21. Thus, parasite eggs deposited between August 21 and September 15, inclusive, produced both transforming and wintering Macrocentrus. The last parasite eggs of the season, the progeny of which survived hibernation and emerged as adults the following spring, were deposited on November 2.

In 1928 it was possible to begin the studies in the insectary with both moths and parasites that emerged from hibernation in outdoor cages. The first Grapholitha eggs were deposited on April 25, and the last eggs from which transforming fruit moths developed were deposited on August 25. Eggs producing wintering Grapholitha were deposited from August 2 to September 11, inclusive. Both transforming and wintering Grapholitha developed from eggs deposited from August 2 to August 25, inclusive.

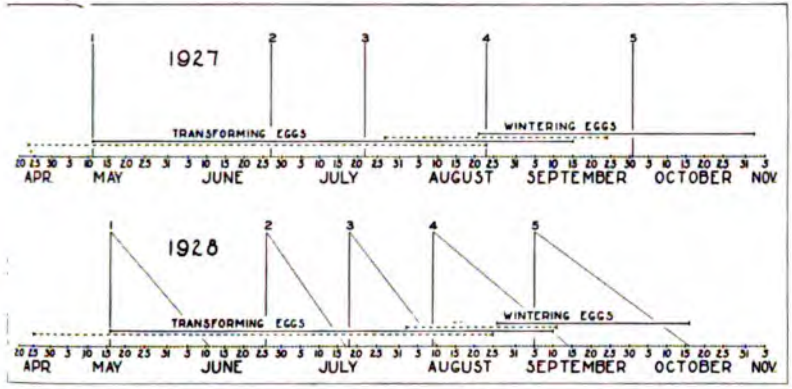

FIGURE 12.—Comparative periods during which eggs were deposited by *Macrocentrus ancylivorus* (solid line) and *Grapholitha molesta* (dotted line) and number of generations of *M. ancylivorus* in 1927 and 1928. The period of egg deposition for each generation of parasites in 1928 is indicated by the slanting line which reaches the base on the date the last egg was laid. The beginning of each generation is marked by a vertical line with the number of the generation at the top

Eggs that produced transforming parasites in 1928 were deposited from May 16 to September 10, inclusive, and eggs producing wintering parasites were deposited from August 26 to October 16, inclusive. Both transforming and wintering Macrocentrus developed from parasite eggs deposited between August 26 and September 10, inclusive.

NUMBER OF GENERATIONS

Five generations of *Macrocentrus ancylirorus* occurred during each of the seasons of 1927 and 1928. The dates on which the first eggs of each brood were deposited during each season are indicated in Figure 12. The period of egg deposition for each brood in 1928 is also shown, being indicated by the slanting line terminating at the date on which the last egg was laid. Similar records were not available for 1927.

In 1927 the first and second broods were entirely transforming. All of the third-brood eggs, except a few of the latest ones deposited, produced transforming parasites. About two-thirds of the eggs of the fourth brood produced transforming individuals, and the remainder produced wintering ones. The fifth brood was entirely a wintering one. There were only four generations of the host species during that season.

In 1928 the first three broods of parasites were entirely transforming. Brood 4 contained a decided majority of transforming individuals, and brood 5, except for a few from the very earliest eggs that were deposited, produced wintering individuals. A period of about two weeks elapsed between the time of deposition of the last eggs of the first brood and the first eggs of the second brood in 1928. This was due to the fact that practically all the female parasites which emerged in the spring from hibernating material had died by the middle of June, and as an average life cycle of 41.3 days was required for development of the earliest individuals of the first brood, eggs of which were deposited on May 16, first-brood adults did not begin to emerge until about June 26. Thus, female parasites were not present in the insectary to deposit eggs from June 11 to June 26. A decided decrease in parasitism by *Macrocentrus ancylivorus* also occurred in the field during this same period in 1928, as indicated by weekly collections of Grapholitha larvae from infested peach twigs. These data have been presented in tabular form by the writer (*3, p. 373*). Parasitism by this species, which had reached a peak of 49.9 per cent in the field at the collection of May 29–June 1, gradually decreased to a minimum of 17.9 per cent for the period June 19–20. It then increased to 29.5 per cent for the period June 26–28, and the following week, July 3–5, it jumped to the high peak of the season, 67.9 per cent. Since the twigs were heavily infested by larvae of the host species throughout this entire period it is evident that the decrease in parasitism in the field was also due to a scarcity of female parasites during the two weeks immediately preceding the emergence of first-brood adults late in June. Five complete or partial generations of the host species occurred in 1928.

HIBERNATION

Macrocentrus ancylivorus hibernates as a first-instar larva within the body of the full-grown hibernating host larva. In the spring of 1929, a few weeks before wintering parasite larvae would begin to emerge from the host larvae, a number of hibernating larvae of the oriental fruit moth that had been parasitized by *M. ancylivorus* the previous fall were dissected. Each Grapholitha larva contained one live first-instar parasite larva and, in most instances, also several head capsules and bodies of dead parasite larvae. Measurements of the head capsules of the dead parasite larvae showed them to be of the same instar as the live ones.

During both 1927 and 1928 a considerable number of parasite larvae reached the cocoon stage late in the fall but failed to emerge as adults. Such cocoons were allowed to go through the winter undisturbed in order to ascertain whether any individuals might successfully hibernate within the parasite cocoon. In no instance did adults emerge in the spring from parasite cocoons spun the previous fall. The majority of these cocoons, when opened, were found to contain dead pupae, which suggests that these individuals pupated late in the fall but were unable to survive the sudden drops in temperature which occurred before they could emerge.

Considerable mortality after the parasite cocoon stage was reached also occurred in parasites which survived hibernation within the host and reached the cocoon stage in the spring. In 1928 and 1929 50 and 63 per cent, respectively, of the hibernating parasites that emerged from the host and spun cocoons in the spring failed to emerge as adults. Subsequent examination of these did not reveal the cause of death, although it was found that the majority had died as prepupae.

SUMMARY

Macrocentrus ancylivorus Roh. is an important parasite of the oriental fruit moth in the vicinity of Riverton, N. J. A closely related species, *M. delicatus* Cress., is parasitic on the same host species, but adults of *ancylivorus* and *delicatus* have distinguishing characters by which living specimens may be separated.

A greater percentage of female than of male parasites is produced when larvae of the oriental fruit moth and larvae of the strawberry leaf roller are parasitized by *Macrocentrus ancylivorus* in the field. A consistently greater proportion of females does not always occur when parasitism takes place under insectary conditions.

The average longevity of adult parasites of both sexes confined together in cages is 6.7 days; the average for unfertilized females confined alone is 17.9 days, and for males confined alone, 10.3 days.

Oviposition is possible only when the larva of the host species is in a position in which it can not escape the ovipositor of the female parasite. Superparasitism occurs, but only one parasite larva survives.

Unfertilized females produce only males.

Fruit-moth larvae of all sizes are subject to attack by this parasite. Parasitism in the field occurs chiefly in larvae of the early broods which feed in peach twigs.

As many as 40 offspring have been produced by a single female of *Macrocentrus ancylivorus* under insectary conditions.

The transforming parasite larva emerges from the host, on an average, 3 days after the Grapholitha larva would normally have pupated, and feeds on it for 12 to 18 hours. Wintering parasite larvae emerge from the host larvae in the spring and require an average of 2 days for feeding. About 24 hours are required for formation of the parasite cocoon. The combined prepupal and pupal period averages 15.2 days for transforming parasites and 38.5 days for wintering parasites. The average life cycle of transforming and wintering Macrocentrus is, respectively, 5.1 and 31 days shorter than the average life cycle of transforming and wintering Grapholitha.

Both adult parasites and moths emerge earlier in the spring from larvae which hibernate in outdoor cages than from larvae which hibernate in the insectary. In outdoor cages the spring emergence of the moths occurs considerably earlier than the spring emergence of the parasites.

During late summer and early fall there is a period during which eggs deposited by Macrocentrus and by Grapholitha may produce both transforming and wintering progeny.

There were five generations of this parasite in the Riverton-Moorestown area in 1927 and in 1928.

Macrocentrus hibernates as a first-stage larva within the full-grown hibernating larva of the oriental fruit moth. Many parasite cocoons that are constructed late in the fall and in the spring fail to produce adult parasites owing to mortality of the prepupae and pupae.

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van den Schrijver

Aanteekening omtrent muskieten (II),

DOOR

Dr. J. HAGA.

(Centraal Militair Geneeskundig Laboratorium.)

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Aanteekening omtrent muskieten (II) 1),

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Dr. J. HAGA.

(Centraal Militair Geneeskundig Laboratorium.)

I. Anophelinen:

A. NIEUWE VINDPLAATSEN.

Een opgave van de vindplaatsen van Anophelinen, die nog niet vermeld staan in den tweeden druk van SWELLENGREBEL's monographie, evenmin als in de vorige opgave 1) over 1922, volgt hieronder.

Myzorhynchus sinensis: Java: Tjitjoeroeg, Gombong, Sumatra: Blang Pidie, Blang Kedjeren, Takengon, Sidikalang, Moeara Tebo; Borneo: Bandjermassin; Celebes: Mamoedjoe.

Myzorhynchus barbirostris: Java: Gombong, Magelang; Sumatra: Moeara Tebo, Sidikalang, Rau (Padang), Langsa; Alor: Kalabahi.

Myzorhynchus barbirostris var. pallidus: Sumatra: Sidikalang.

Myzorhynchus albotaeniatus: Sumatra: Meulaboh, Moeara Tebo.

Myzorhynchus umbrosus: Sumatra: Moeara Tebo.

Myzorhynchus novumbrosus: Sumatra: Moeara Tebo.

Stethomyia aitkeni var. ins. florum: Alor: Kalabahi.

Myzomyia ludlowi: Java: Ambarawa; Sumatra: Bireuen; Alor: Kalabahi; Ceram: Piroe; Ternate.

Myzomyia rossii Java: Soemedang, Gombong, Magelang; Sumatra: Lokop; Celebes: Mamoedjoe; Alor: Kalabahi.

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1) Vervolg op het gelijkluidende artikel, verschenen in het *Geneeskundig Tijdschrift voor Nederlandsch-Indie*, afl. 4, Deel 63.

Myzomyia vaga: Java: Soemedang, Gombong [1]), Malang; Poeloe We: Sabang; Sumatra: Blang Pidie, Sidikalang; Padang, Moeara Tebo; Alor: Kalabahi.

Myzomyia aconita: Java: Soemedang, Gombong, Magelang; Sumatra: Blang Kedjeren; Alor: Kalabahi.

Myzomyia minima: Java: Gombong; Alor: Kalabahi.

Cellia kochii: Java: Magelang; Billiton; Sumatra: Langsa, Lokop, Sidikalang, Moeara Tebo.

Neomyzomyia tesselata, Theo. (= *Neomyzomyia punctulata* Swellengrebel): Java: Magelang: Sumatra: Moeara Tebo.

Neomyzomyia leucosphyra: Sumatra: Moeara Tebo.

Neomyz. leucosphyra var. hackeri: Sumatra: Moeara Tebo; Celebes: Mamoedjoe.

Nyssorhynchus maculatus: Java: Noesakambangan; Sumatra: Blang Kedjeren, Takengon, Sidikalang; Alor: Kalabahi.

Nyssorhynchus fuliginosus: Java: Gombong, Ambarawa, Magelang; Sumatra: Lokop, Blang Kedjeren, Moeara Tebo.

Nyssorhynchus jamesi: Sumatra: Moeara Tebo.

B. EENIGE BIJZONDERHEDEN BETREFFENDE ENKELE SOORTEN.

Myzorhynchus sinensis: Reeds vroeger werd opgemerkt, dat bij deze soort de banden op de palpen vaak onduidelijk zijn en dan ternauwernood den naam „banden" mogen dragen [2]). Echter vertoonen vele exemplaren van het *type* slechte twee (nl. de het meest apicaal gelegen) witte palpringen, die dan bovendien nog dikwijls onduidelijk zijn. Evenwel komt het ook voor, dat die 2 witte topringen zoo breed worden, dat ze met elkaar versmelten, waardoor dan één breede apicale band ontstaat.

De *vleugelteekening* laat soms maar een *kleine* lichte costaplek, bij de inmonding der subcosta, zien, welke niet op de

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[1]) Daaronder begrepen: Dapoen en Petemon.

[2]) Ziet: Extract uit het Jaarverslag van het Centraal Militair Geneeskundig Laboratorium over het jaar 1921, *Geneesk. Tijdschr. v. Ned.-Indië*, afl. 3, Deel 62, 1922, p. 339.

1ste long. overgrijpt, hetgeen volgens SWELLENGREBEL kenmerkend is voor de *var. vanus*, terwijl dan toch de lichte banden op de tarsi der achterpooten smal zijn en daarin dus overeenkomen met de teekening bij het *type*.

Myzorhynchus barbirostris en *Myzorhynchus barbirostris var. pallidus*: De *Myzorh. barbirostris* blijkt aan eenige variaties onderhevig te zijn, te oordeelen naar de exemplaren in de verzameling van het Centraal Militair Geneeskundig Laboratorum die interessante verschillen opleveren.

Als het *type* van den *M. barbirostris* willen we die soort beschouwen, waarbij de ventrale gedeelten der segmenten 2-7 van het abdomen der imagines witte mediane schubben (ter weerszijden vlak naast de middellijn) vertoonen.

De daarop voorkomende *variaties* willen we nu kort beschrijven:

1. Bij de meest voorkomende variatie vinden we, behalve de mediane ventrale witte schubbundels, ook fraaie laterale witte schubben op de abdominale segmenten 2-7, in één rij of eenige (2-3) lengte-rijen. (Vele exemplaren van Weltevreden, Gombong en Magelang (Java), Lokop en Sidikalang (Sumatra) en Mamoedjoe (Celebes)) . . . . varietas 1.

2. Abdominale schubben teekening als onder 1 beschreven, maar bovendien op het ventrale basale deel van het 8ste segment, ter weerszijden der mediaanlijn (en distaal van de beide zwarte schubbenbundels op den top van het 7de abd. segm.) een bundel goudgele schubben. (Het exemplaar met deze variëteit (N°. 3443) is afkomstig van Mamoedjoe (Celebes) en heeft bovendien nog een afwijkende pootenteekening: de femora en tibiae namenlijk zijn door de vele er op aanwezige witte schubben fraai gespikkeld. Bij het *type* zijn de pooten (behalve de lichte banden) donkerbruin . . . . varietas 2.

3. Abdomen ventraal zonder witte schubben, noch mediaan, noch lateraal, op 2de-7de segment . . . . varietas 3.

Van deze variëteit bezit het C.M.G.L. twee exemplaren, nl. een ♀ exemplaar van Amboina (N°. 125) en een ♂ exemplaar van Boelongan (Borneo, N°. 2314). Beide vertoonen het typische *barbirostris-kenmerk* van RODENWALDT (een groep zilverwitte schubben met 3-4 haren op den achterbeugel). Bovendien is van het tweede exemplaar tevens de larvehuid in canadabalsem gemonteerd en de prachtige typische volkomen waaiers op de abdominale segmenten 3-7 bewijzen ook, dat deze huid niet van een umbrosus-larve afkomstig kan zijn. Dat ze evenmin afkomstig is van den *M. barb. v. pallidus* bewijzen de zijclypeusharen, waaraan we een 45-50 takjes hebben kunnen tellen (de larve der *var. pallidus* heeft immers zijclypeusharen met 17 of minder takken). Dit laatste kan, wegens het gemis van de larvehuid, niet bewezen worden voor het ♀ exemplaar van Amboina, waar, zooals bekend, ook de var. pallidus voorkomt.

In aansluiting aan deze 3de variëteit moeten we den *M. barbirostris var. pallidus* bespreken.

SWELLENGREBEL ¹) zegt hiervan:

„De imago dezer variëteit is duidelijk kleiner dan die van het type, de lichaamslengte der eerste is niet veel meer dan de helft van die van het laatste. De kleur is ook duidelijk lichter. Waar men bij barbirostris aan den vleugeltop een lichte plek vindt aan de inmonding der derde long., is deze bij de var. pallidus doorgetrokken van af de inmonding van den ondertak der 2de long. tot aan die van den boventak van de 4de long. De lichte costaplek bij de inmonding der subcosta is bij de variëteit grooter dan bij het type".

De twee in de verzameling aanwezige (♂ en ♀) exemplaren, afkomstig van Noesakambangan (ontvangen van Prof. RODENWALDT), zijn wel duidelijk kleiner dan het type, maar het zijn uit larven gekweekte en na het opprikken, voor zoover het abdomen betreft, sterk geschrompelde exempla-

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¹) Ziet: De Anophelinen van Nederlandsch-Oost-Indië, 2de dr 1921 (p. 120), en *Geneesk. Tijdschr. v. Ned.-Indië*, 1919, Deel 59, 1ste afl. Eenige voor Nederlandsch-Indië nieuwe Anophelinen, p. 8-9.

ren. Daarentegen beschikt het Centraal Militair Geneeskundig Laboratorium ook over 27 exemplaren van Sidikalang (15 ♂ en 12 ♀), bijna alle door den Off. v. Gez. J. TJAKKES uit larven gekweekt, welke laatste door hem als *var. pallidus* gedetermineerd waren. Vele zijn ook kleiner dan het *type*, indien we de exemplaren daarvan in de natuur vangen, *bijna de helft (13) echter even groot of nagenoeg even groot als het type.*

Wat nu de grootte betreft, moeten we opmerken, dat het bekend is, dat bij het kweeken uit larven van eenzelfde soort zeer verschillend groote imagines kunnen verkregen worden, al naar gelang de larven reeds hun volkomen wasdom bereikt hadden, dan wel zich, door het verplaatst worden in onnatuurlijke en ongunstigere omstandigheden, voortijdig verpoppen en dientengevolge als veel kleinere imagines uitkomen.

Waar bij de beschrijving van de imago van de *M. barb. v. pallidus* SWELLEGREBEL niets opmerkt over het abdomen, zou verondersteld kunnen worden, dat de *pallidus* óók witte schubben op de ventrale abdominale segmenten 2-7 zou vertoonen. Bij de twee exemplaren van Noesakambangen viel echter terstond het *totaal ontbreken der witte schubben* op. De heer RODENWALDT bleek dit eveneens reeds opgemerkt te hebben bij al zijn, uit larven gekweekte, exemplaren van Noesakambangan.

Bij alle 27 uit Sidikalang ontvangen imagines, welker larven door Dr. TJAKKES als *barbirostris var. pallidus* gedetermineerd waren, *ontbraken eveneens de witte schubben op de ventrale abdominale segmenten geheel.* Dat in Sidikalang de echte var. pallidus voorkomt, bewijzen voorts de vele vandaar in December 1923 ontvangen typische larven, wier zijclypeusharen een tiental [1]) takken vertoonen, terwijl de middenclypeusharen vlak bij elkaar staan. Daarnaast komt

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[1]) Dit aantal wisselt bij de door ons daarop nagekeken exemplaren tusschen 8 en 15.

in Sidikalang ook de *barbirostris* voor, mogelijk het *type* (waarvan we echter nog geen exemplaren hebben ontvangen), maar zeker de boven beschreven varietas 1 (met *mediane en laterale* witte schubben op 2de-7de abdominale segmenten).

De bestudeering van 20 geheel onbeschadigde exemplaren uit Sidikalang (10 ♂ en 10 ♀) en van het eene onbeschadigde ♂ exemplaar van Noesakambangan leverde het volgende op:

1°. bij bijna de helft der exemplaren was de *grootte* gelijk aan die van het type (ziet ook p. 5);

2°. bij geen der exemplaren is de *kleur* duidelijk lichter dan die van het type, integendeel, volkomen gelijk te noemen;

3°. wat betreft de teekening van den vleugeltop:

a. constant aanwezig (bij alle 21 exemplaren) *was de lichte franjeplek bij de inmonding van den boventak der 2de long.*

(Op dit kenmerk werden talrijke barbirostris-exemplaren (type en var. 1-3) nagekeken, maar deze vertoonden slechts in een minderheid der gevallen deze lichte plek.) Alleen was de grootte der plek verschillend; bijna steeds was ze vrij smal en duidelijk door een zwart franjegedeelte gescheiden van de daar boven gelegen lichte plek bij de inmonding van de 1ste long. in de costa. Bij twee ♂'s echter waren deze beide plekken bijna versmolten, door verbreeding der plek in kwestie.

b. de *lichte plek bij de inmonding der 3de long.* wisselt in grootte: ze strekt zich nl. uit:

α. van de inmonding der 3de long. tot de inmonding van den boventak der 4de long. in de costa bij 8 exemplaren, d.i. 38%;

β. van even voor de inmonding der 3de long. tot de inmonding van den boventak der 4de long. in de costa bij 9 exemplaren, d.i. 43%;

γ. van de inmonding van de 3de long. tot voorbij de inmonding van den boventak der 4de long. (nl. tot midden tusschen den boven- en ondertak der 4de long.) bij 3 exemplaren, d.i. 14%;

δ. over de inmondingsplaats der 3de long. bij 1 exemplaar (♂) d.i. 5°/₀.

De onder α, β en γ vallende exemplaren komen dus goed overeen met het als normaal (ziet boven) door SWELLENGREBEL voor de *var. pallidus* beschrevene, en hiertoe behooren dan ook bijna al onze exemplaren (20 van de 21). Slechts één exemplaar geeft een teekening te zien, gelijk die bij het *type* voorkomt.

c. de *lichte costa-plek bij de inmonding der subcosta* is dikwijls even smal als bij het *type*.

Uit het bovenstaande willen we slechts besluiten, dat het ons nu onmogelijk is geworden om met volkomen zekerheid de *imagines* van *barb. var. pallidus* te onderscheiden van die der var. 3 (ziet boven). Waar de grootte, kleur, teekening van abdomen en vleugel geheel kunnen overeenkomen, zal men het vraagstuk van een anderen kant dienen op te lossen, nl. door zooveel mogelijk barbirostris-larven te verzamelen en onder zoo gunstig mogelijke omstandigheden uit te broeden, zoodat we naast de imagines ook de daarmede correspondeerende larvehuiden bewaren kunnen. Het is mogelijk, dat dan blijken zal dat de lichte plek bij de inmonding van den boventak der 2de long. constant of nagenoeg constant aanwezig is bij de *var. pallidus*, en veel zeldzamer bij het *type* zal voorkomen.

Dat de *var. pallidus*, op grond van den van het *type* afwijkenden bouw der zijclypeusharen in het larve-stadium, zeker met meer recht dan de bovengenoemde andere variëteiten als echte variëteit opgevat kan worden, willen we ook niet betwijfelen. Gemakshalve hebben we de bovengeschetste afwijkingen bij de imagines als variëteiten opgenoemd, zonder te willen beweren, dat ook reeds in het larve-stadium afwijkingen zouden voorkomen.

De imagines der 3 variëteiten en de 29 exemplaren van de *var. pallidus* hebben we bovendien nog nagekeken op het door RODENWALDT voor den *M. barbirostris* als *typisch* *kenmerk* aangegeven voorkomen van *zilverwitte schubben* (van 6-20) en *3-4 haren op den achterbeugel.* Evenals voor het type ging ook voor deze exemplaren het kenmerk zonder uitzondering op. Daar nu het door SWELLENGREBEL in zijn monographie (1921, p. 116) aangegeven „beslissend kenmerk: witte schubben ventraal op abdominaal segment 2-7" bij de imagines der *var.* 3 en *var. pallidus* ontbreekt, terwijl RODENWALDT's kenmerk nooit in den steek laat, komt het ons noodzakelijk voor, dit laatste kenmerk het eerst te noemen, en daarna als tweede te vermelden, dat op de ventrale gedeelten der abdominale segmenten 2-7 *nagenoeg steeds* witte schubben (alléén mediaan òf mediaan èn lateraal) voorkomen. Bovendien moet bij de beschrijving der imago van den *M. barb. var. palildus* uitdrukkelijk geconstateerd worden, dat deze *abdominale witte schubben steeds, daarentegen de schubben en haren op den achterbeugel nooit ontbreken,* terwijl de *grootte* en *kleur* der imago *gelijk* kunnen zijn aan die van het *type.*

Verder missen de volgende soorten, waarvan zulks in SWELLENGREBEL's monographie niet vermeld is, de schubben op den achterbeugel geheel:

Myzomyia minima,
Nyssorhynchus jamesi,
Nyssorhynchus schüffneri, en de
Stethomyia aitkeni var. insulae florum.

De *Myzorhynchus gigas* en de *Stethomyia aitkeni type* ontbreken nog in de verzameling en konden dus op dit kenmerk niet nagekeken worden.

Mzyomyia ludlowi: Deze vertoont bij onze exemplaren meermalen van het *type afwijkende vleugelteekeningen.* Vaak zijn de 1ste en 2de groote costavlek (gerekend van af de vleugelbasis) versmolten (exemplaren van Bireuen). Zeer dikwijls komt de vergrooting van de vlekken voor, o.a. op den boventak der 5de long. (door samenvloeiïng der 2 ap. vlekken), zoodat deze nagenoeg geheel zwart is. Verder wijkt de *palpenteekening* wel eens van de norm af. SWELLENGREBEL zegt immers (l. c., p. 44): „Palpen donkerbruin, de lichte top even lang, *hoogstens 2 × zoo lang* als de zwarte ring er onder." (cursiveering van mij, H.). Nu vertoont een ♀ *Myzomyia ludlowi* van Koeta-Radja (N°. 2036) een lichten palpentop, die 2½ × zoo lang is als de daaronder gelegen zwarte ring, terwijl een ♀ exemplaar van Piroe (Ceram, N°. 5098) deze afwijking nog overdrijft, door op beide palpen een distalen witten top te vertoonen, die 3 × zoo lang is als de daaronder gelegen zwarte ring. Beide exemplaren bezitten verder normaal gevlekte pooten en zijn dus zeker niet tot de *var. flavescens Sw.* te rekenen.

Myzomyia rossii: De *alpen teekening* hierbij is soms ook afwijkend. Eenige ♀ exemplaren, afkomstig van Pare-Pare (Celebes, N°. 684, 685, 686) bezitten nl. een vrij duidelijken, overtolligen (vierden) palpband, doordat het bruinzwarte gedeelte, gelegen tusschen den lichten apicalen en den subapicalen band, een smallen ring van witte schubben vertoont.

Myzomyia vaga: Behalve de reeds vroeger (ziet noot, p. 1) opgenoemde afwijkingen in de *palpteekening*, komt nog een variant veelvuldig voor. Dikwijls is nl. de lichte palpentop niet 3-5 maal zoo breed als de daaronder gelegen zwarte ring (SWELLENGREBEL), maar *slechts 2 maal* of *nog niet eens 2 maal*, hetgeen vele exemplaren van Gombong vertoonen (N°. 4187, 4197, 4206, 4245, 4350, 4351, 4431 t/m 4435). (Daarentegen kan de lichte palpentop ook buitenmatig lang zijn, wel 10-11 maal zoo lang als de daaronder gelegen zwarte ring: o. a. Gombong N°. 4291, 4393). De proboscis vertoont echter bij al deze exemplaren steeds gelukkig de lichte plek aan de proboscis (proximaal van de oliva), die zoo karakteristiek is voor de *vaga*, wegens het absoluut ontbreken ervan bij de *Myzomia rossii* en *M. ludlowi*. Soms, doch uiterst zelden, ontbreekt ook bij de *M. vaga* (die in die gevallen gelukkig weer een typische palpteekening had) deze lichte plek (ziet: *Geneesk. Tijdschr. v. Ned.-Indië*, afl. 3. Deel 62, 1922, p. 339).

Stethomyia aitkeni var. insulae florum: SWELLENGREBEL zegt van de imago dezer variëteit (l.c., p. 136): „Als voorgaande soort, alleen mannelijke genitalia verschillen", zoodat zij voor het overige dus met de imago van het *type* moet overeenkomen. Over den vleugel van het ♀ zegt SWELLENGREBEL nu o.a. het volgende (l. c., p. 134 en 135):

1. „Stam 2de long. minder dan 2/5 van de lengte der eerste vorkcel."

2. „De 3de long. loopt, in tegenstelling van den gewonen toestand bij de anophelinen, niet basaalwaarts van de voorste dwarsader door, maar eindigt in deze . . . . ".

Het mag dan ook toevallig heeten, dat het eerste ♀, dat we van deze soort van Kalabahi (Alor) ontvingen (geschenk van Prof. RODENWALDT), op beide punten aanzienlijke afwijkingen opleverde. Het ♂ gaf ongeveer hetzelfde te zien, maar daar SWELLENGREBEL niets over den vleugelbouw der mannetjes vermeldt, terwijl deze dikwijls, zoowel bij Anophelinen als Culicinen, wat den vleugel betreft, aanzienlijke verschillen met de wijfjes vertoonen, valt hieruit geen besluit te trekken. Toen we echter nog 11 (7 ♂ en 4 ♀) exemplaren uit Kalabahi, van Off. v. Gez. K. A. AARSTAD, ontvingen, hebben we ook deze op genoemde kenmerken nagezien en de resultaten daarvan in onderstaande tabel neergelegd.

STAAT.

Stethomyia aitkeni var. insulae florum (Kalabahi). [1]

♂				♀			
No.	Lengte stam 2de long.	Lengte 1ste vork-cel.	3de long. voorbij voorste dwars-ader?	No.	Lengte stam 2de long.	Lengte 1ste vork-cel.	3de long. voorbij voorste dwars-ader?
2874	13.	25.	ja, nl.= 1/3 lengte v. dw. ad.	2873	19,5.	37,5.	ja, nl.= lengte van v.&m.dw. ad. samen.
5218	14.	27.	neen.				
5219	14.	24.	ja, nl = lengte v. dw. ad.	5227	15.	35.	neen.
				5228	16.	38.	neen.
5221	13.	25.	neen.	5229	14.	32.	neen.
5222	15.	26,5.	ja, nl.= lengte v. dw. ad.	5230	13.	32.	neen.
5223	14.	26.	heel even.				
5224	12.	26.	neen.				
5225	11.	23	heel even.				

Deze uitkomsten kunnen we dus als volgt samenvatten:

1. *a. Bij de ♂ 's* is de *gemiddelde verhouding* van de lengte van den stam der 2de long. tot de lengte der 1ste vorkcel als 92:175 = 52:100, of *vrijwel = 1:2;*

b. bij de ♀ 's is die verhouding als 77,5:174,5 = 44:100 of *iets meer dan 2/5.* Bij het ♀ N°. 2873 alléén is de bewuste verhouding als 19,5:37,5 = 52:100, dus als de gemiddelde verhouding bij de ♂ 's. Bij de overige 4 ♀ 's is de gemiddelde verhouding als 58:137 = 42:100, dus toch nog *iets meer dan 2/5.*

2. *a. Bij de ♂ 's* loopt de 3de long., in mindere of meer-

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[1]). De lengten van den stam der 2de long, resp. 1ste vorkcel bij de verschillende exemplaren zijn uitgedrukt in getallen, weergevende het aantal streepjes der schaalverdeeling van Zeiss' meetoculair 3, aangewend in Zeiss' binoculair microscoop met het objectief-paar a 3 (Vergr. 50 ×).

dere mate, ongeveer in de helft der gevallen bassalwaarts voorbij de voorste dwarsader door:

b. bij de ♀ *'s* loopt de 3de long. meestal niet voorbij de voorste dwarsader door, zooals SWELLENGREBEL ook aangeeft.

Karakteristieke kenmerken voor de imagines van enkele soorten van Nederlandsch-Oost-Indische Anophelinen.

Het onderstaande moet beschouwd worden als een aanvulling op de door SWELLENGREBEL aangegeven karakteristieke kenmerken (l.c., p. 29-30).

Proboscis: *a.* donker, maar proximaal van de oliva een lichte plek, of ventraal, of dorsaal, of circulair: *Myzomyia vaga* ♀.

b. distale helft (dorsaal èn ventraal) licht bij de ♀ *'s van 4 soorten: Myzomyia aconita, Cellia kochii, Nyssorhynchus punctulatus Dönitz* (nec Sw.) en *Neomyzomyia tessellata* (= Neom. punctulata Sw.), en bij de ♂ *'s van 2 dier soorten,* nl. *Cellia kochii* en *Nyssorhynchus punctulatus Döntz.*

c. geheel roomwit: *Myzomyia immaculata* (♂ en ♀).

Palpen: a. geheel bronsachtig bruin, ongeband, even lang als het abdomen: *Stethomyia aitkeni,* ♂ en ♀ (*type* èn *var. insulae florum*).

b. geheel zwart alleen bij de ♀ 's van 3 soorten en zwart of bruin en zwart bij de ♂ 's er van: *Myzorhynchus albotaeniatus, Myzorhynchus barbirostris* en *Myzorhynchus umbrosus* (bij *M. albotaeniatus* ♀ soms enkele geelbruine schubben op de grens van 2de-3de en 3de-4de lid).

c. geheel roomwit (evenals proboscis) bij het ♀, en aan den top wat geler bij het ♂ van *Myzomyia immaculata.*

d. met wit-geel-zwarte kleurschakeeringen van den top naar de basis der palpi alleen bij *Cellia kochii,* en wel als volgt bij het:

♀: geel-zwart; wit-geel-zwart; wit-geel met zwart-zwart; wit-geel met zwart-zwart met geel;

♂: geel-zwart; (wit)-geel-zwart; geel-zwart; geel-zwart; geel-zwart-geel.

Thorax: Achterbeugel: met 3-4 haren en een groepje van zilverwitte, naar voren en naar boven gerichte platte schub- (8-20) (RODENWALDT): bij ♂ en ♀ van het *type en alle variëteiten*, inbegrepen de *var. pallidus* van *Myzorhynchus barbirostris.*

Abdomen: rijen van witte schubben mediaan (en lateraal) op de ventrale zijde van 2 of meer (6) abdominale segmenten (2de-7de): *Myzorhynchus barbirostris*, ♂ en ♀.

N.B.: Dit kenmerk ontbreekt steeds bij den *Myzorhynchus barbirostris var. pallidus* en voorts bij *nog een variëteit* van den *Myzorhynchus barbirostris* (ziet echter het kenmerk van den achterbeugel!).

II. Culicinen:

A. BESCHRIJVING DER VOLLEDIGE VERZAMELING VAN CULICINEN, MET VERMELDING VAN OUDE EN NIEUW VINDPLAATSEN.

Zooals in de vorige vermelding (zie noot, p. 1) is hier de nomenclatuur volgens EDWARDS gevolgd (*Ind. Journ. of Med. Res.* 1922-1923, X, p. 249-293 en 430-475).

Het bleek bij herziening van het materiaal en bij onderzoek door den entomoloog. van het *British Museum* Mr. F. W. EDWARDS, van vele, door den dir. off. v. gez. 1. kl. S. L. BRUG naar Londen medegenomen exemplaren, zoowel van imagines als larven, dat sommige soorten niet juist gedetermineerd waren.

Daar een volledige opgave der verzameling nog niet gepubliceerd is en de veranderingen, in de vorige opgaven aangebracht en hierboven vermeld, tot verwarringen aanleiding kunnen geven, moge zij hieronder volgen, waarbij om dezelfde redenen alle vindplaatsen (ook van de vorige jaren) vermeld zijn. De *nieuwe vindplaatsen of in 1923 bijgekomen nieuwe soorten* zijn aangegeven door vóór de vindplaatsen en achter de soort (1923) te plaatsen.

MEGARHINUS:

splendens: Poeloe We: Sabang; (1923) Sumatra: Koeta-Radja.

URANOTAENIA:

atra (s. *cancer,* Leic.): Eiland Edam; Java: Weltevreden, Soerabaja; (1923) Sumatra: Lokop.

bimacuiala: Borneo: Boelongan; (1923) Java: Weltevreden.

brevirostris: (1923) Sumatra: Moeara Tebo.

campestris: (1923) Sumatra: Moeara Tebo.

metatarsata: (1923) Java: Weltvreden.

micans: (1923) Java: Weltevreden.

papua: Nieuw-Guinea: Pionierbivak.

sp. n.?: (1923) Alor: Kalabahi.

subnormalis, Martini (= *roperi,* Edw.): Borneo: Tanah Grogot (♀); (1923) Java: Weltevreden (2 ♂, 1 ♀).

RACHISOURA:

atripes, Skuse: (1923) Timor: Atapoepoe.

RACHIONOTOMYIA:

sp. n., (1923) Timor: Atamboea (zal door EDWARDS beschreven worden).

MUCIDUS:

laniger (= *M. mucidus*): Sumatra: Koeta Tjane; (1923) Sumatra: Moeara Tebo.

PARDOMYIA:

aurantia: (1923) Sumatra: Moeara Tebo.

TAENIORHYNCHUS:

(subgenus: *Coquillettidia*):

brevicellulus (= *Chrysoconops pygmaeus,* Theo.): Java: Weltevreden.

giblini (= *T. conopas,* Theo.): Sumatra, Lho Soekon.

ochraceus Theo. (= *Mansonia chrysogona,* Knab; *Chrysoconops aurites,* Theo): Sumatra: Lho Soekon; Riouw-Archipel: Tandjong Pinang; (1923) Java: Weltevreden.

sp. n.?: (1923) Sumatra: Lho Seumawe.

(subgenus: *Mansonioides*):

annuliferus (= *Panoplites annuliferus*, Theo.; *Mansonia septempunctata*, Theo.; *Mansonioides septemguttata*, Theo.): Sumatra: Lho Soekon; (1923) Moeara Tebo.

annulipes: Riouw-Archipel: Tandjong Pinang; Nieuw-Guinea: Assike, Pionierbivak; Borneo: Singkawang, Samarinda, Bandjermassin, Tanah Grogot, Sampit; (1923) Sumatra: Moeara Tebo; Java: Weltevreden.

uniformis (= *Mansonia africana var. reversus*, Theo.): Nieuw-Guinea: Pionierbivak, Toerai; Celebes: Paloppo; Ceram: Piroe; Sumatra: Padang Sidempoean, Lokop; Java: Weltevreden; (1923) Sumatra: Moeara Tebo.

papuensis, Tayl.: Nieuw-Guinea: Pionierbivak.

AEDES:

(subgenus: *Stegomyia*:)

albolineatus: Borneo: Samarinda.

albopictus (= *Steg. scutellaris*, Theo.): Java Weltevreden, Tjitjoeroeg (Pr. R.); Sumatra: Padang Pandjang, Lokop; Poeloe We; Sabang: Borneo: Samarinda, Bandjermassin, Boelongan; Timor: Koepang; Alor: Kalabahi; Saparoea; Nieuw-Guinea: Manokwari; (1923); Java: Magelang, Dapoen (bij Gombong); Sumatra: Moeara Tebo; Ceram: Piroe.

argenteus, Poiret (= *Steg. fasciata*): Java Weltevreden, Tjitjeureub. (Pr. R.); Eiland Edam: Poeloe We: Sabang; Borneo: Singkawang, Bandjermassin, Samarinda, Tarakan; Celebes: Loewoe, Pare-Pare; Soembawa; Saparoea; Banda; Timor: Koepang; Nieuw-Guinea: Pionierbivak, Fak-Fak; (1923) Java Bandoeng, Soerakarta, Magelang; Sumatra: Moeara Tebo; Amboina: Amboina; Ceram: Piroe; Alor: Kalsbahi.

(subgenus: *Finlaya*):

niveus, Ludl. (= *pseudoniveus*, Theo.) Borneo: Samarinda, Boelongan.

notoscriptus (= *Steg. notoscripta*, Theo.): Saparoea.

poicilia: Java: Tjitjeureb (Pr. R.); (1923) Java: Magelang; Sumatra: Moeara Tebo.

(subgenus: *Ochlerotatus*):

vigilax: Eiland Edam; Madoera: Kali Anget (bij Soemenep).

(subgenus: *Banksinella*):

lineatopennis, Ludl. (= *B. luteolateralis*, Theo., part.): Java: Weltevreden; Sumatra: Lokop; (1923) Java: Gombong; Sumatra: Takengon, Moeara Tebo.

(subgenus: *Ecculex*):

alboscutellatus (= *Lepidotomyia alboscutellata*, Theo.): (1923) Sumatra: Moeara Tebo.

caecus: Java: Weltevreden; Nieuw-Guinea: Merauke (?); (1923) Java: Gombong, Petemon (bij Gombong); Sumatra: Moeara Tebo.

mediolineatus: (?) ♀ (1923) Sumatra: Moeara Tebo.

niveoscutella (= *Reedomyia*, Theo): Sumatra: Lokop, Bireuen.

vexans: Java: Weltevreden; Madoera: Kali Anget; Borneo (W. Afd.): Ngabang; Celebes: Pare-Pare, Bangsai; Ternate; Timor: Koepang; (1923) Java: Gombong, Petemon; Sumatra: Moeara Tebo; Ceram: Piroe.

(subgenus: *Skusea*):

amesi, Ludl. (= *Steg. fusca*, Leic.): Java: Weltevreden; Borneo: Samarinda, Tarakan,

cancricomes: Eiland Edam.

umbrosus: Borneo: Tanah Grogot; (1923) Java: Weltevreden

(subgenus: *Aedes, s. str.*):

butleri? (= *Skusea diurna*, Theo.) (alleen ♀'s): Celebes: Loewoe; Ceram: Piroe; Saparoea; (1923) Java: Weltevreden, Gombong; Sumatra: Moeara Tebo.

sp. n.? (alleen ♀'s): Nieuw-Guinea: Pionierbivak.

uncus-groep (1 ♀): (1923) Sumatra: Moeara Tebo.

singularis? (1 ♀) (= *Aioretomyia singularis*, Leic.): (1923)

Sumatra: Moeara Tebo.

(subgenus: *Pseudoskusea*):

similis, Theo.: Saparoea; (1923) Amboina.

(subgenus: *incertum*):

ostentatio (= *Aioretomyia ostentatio*, Leic., *Pseudohowardina chrysoscuta*, Theo.): Sumatra: Moeara Tebo.

ARMIGERES: (= *Desvoidya*):

(subgenus: *Armigeres*):

confusus: Borneo: Samarinda.

malayi (= *Desv. fusca*, Theo.;? *Neosquamomyia breinli*, Tayl.): Sumatra: Lokop; Alor: Kalabahi; Saparoea; (1923) Java: Weltevreden.

obturbans: Java: Weltevreden; (1923) Java: Gombong, Petemon (bij Gombong); Sumatra Moeara Tebo; Alor: Kalabahi.

(subgenus: *Leicesteria*):

dolichocephalus (♀): (1923) Sumatra: Lokop.

AEDEOMYIA:

catasticta, Knab *squamipennis*, Theo., Leic., part.): Java: Weltevreden.

ORTHOPODOMYIA:

maculipes: Borneo: Samarinda.

FICALBIA:

luzonensis: (1923) Java Weltevreden.

LUTZIA:

fuscana (= *Culex concolor*, Theo.,): Java: Weltevreden, Bandoeng, Sumatra: Lokop: Borneo: Samarinda; Celebes: Pare-Pare, Paloppo; (1923) Java: Gombong, Petemon (bij Gombong).

halifaxi (= *C. multimaculosus*, Leic.): Nieuw-Guinea: Manokwari, Toerai; Borneo: Samarinda; (1923) Java: Weltevreden; Sumatra: Moeara Tebo; Ceram: Piroe.

CULEX:

(subgenus: *Culex, s. str.*):

bitaeniorhynchus: Java: Weltevreden, Tjitjeureub, Tasikmalaja; Celebes: Pare-Pare (1923) Java: Soemedang, Gombong, Magelang, Malang; Sumatra: Moeara Tebo, Padang, Lokop, Blang Kedjeren; Alor: Kalabahi.

brevipalpis (= *Melanoconion uniformis*, Leic.): Borneo: Samarinda; (1923) Java: Weltevreden; (brevipalpis-groep): Ceram: Piroe. (2 ♀ 's).

fatigans: Eiland Edam; Java: Weltevreden, Tjitjeureub, Bandoeng, Tasikmalaja, Soerabaja; Madoera: Kali Anget; Sumatra: Padang Pandjang, Tapa-Toean, Bireuen; Borneo: Bandjermassin, Tanah Grogot, Tarakan, Boelongan; Celebes: Makassar, Loewoe, Pare-Pare, Pampanoea, Bangaai; Boeton; Ceram: Piroe; Ternate; Banda; Saparoea; Dobo (Aroe-eilanden); Nieuw-Guinea: Manokwari, Assike, Fak-Fak; Timor: Koepang, Atapoepoe; Alor: Kalabahi; (1923) Java: Soemedang, Gombong [1]), Magelang, Soerakarta, Malang; Sumatra: Moeara Tebo, Padang, Blang Pidie, Lho Seumawe, Takengon; Celebes: Madjene; Amboina: Amboina.

fuscocephalus, (= *uniformis*, Leic.): Java: Weltevreden; Sumatra: Bireuen; Timor; Atapoepoe; (1923) Java: Gombong, Petemon; Sumatra: Moeara Tebo, Blang Kedjeren, Lokop.

gelidus, *var*. *cuneatus*: Java: Weltevreden; Sumatra: Padang Sidempoean; Celebes: Pare-Pare; (1923) Java: Gombong, Petemon, Soerakarta, Malang (? of type?); Sumatra: Padang (? of type?), Moeara Tebo, Lokop, Blang Kedjeren.

————, *type*: (1923) Java: Weltevreden; Sumatra: Moeara Tebo, Lokop, Alor: Kalabahi.

malayi (= *Aedes malayi*, Leic.): Timor: Koepang; (1923) Sumatra: Moeara Tebo.

mimulus (= *C. mimeticus*, Theo., part.): Eiland Edam;

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[1]). Hierbij inbegrepen de drie in de buurt gelegen kampongs: Dapoen, Petemon en Rawahele.

Borneo: Boelongan, Samarinda, Tanah Grogot; (1923) Sumatra: Moeara Tebo.

minimus: Java: Weltevreden, Bandoeng Tasikmalaja; (1923) Java: Gombong; Sumatra: Moeara Tebo.

minor: (1923) Java: Weltevreden.

muticus: Nieuw-Guinea: Manokwari, Pionierbivak; (1923) Amboina.

sinensis: Java: Weltevreden; Tjitoeroeg; Borneo: Sampit: Celebes: Pare-Pare; (1923) Java: Soemedang; Sumatra: Lokop.

sitiens, Edw.: Java: Weltevreden, Soerabaja; Sumatra: Bireuen, Padang Sidempoean; Borneo: Samarinda, Tarakan Celebes: Pare-Pare, Bangaai; Timor: Koepang; Nieuw-Guinea: Merauke, Pionierbivak, Toerai; (1923) Java: Gombong; Sumatra: Padang, Lho Seumawe; Ceram: Piroe.

tritaeniorhynchus, Edw.: Java: Weltevreden, Soerabaja; Sumatra: Bireuen, Sidempoean; Borneo: Sampit, Boelongan, Tanah Grogot; (1923) Java: Gombong, Petemon, Soerakarta; Sumatra: Moeara Tebo, Padang, Blang Pidie, Lokop, Lho Seumawe.

vishnui, Edw.: Java: Weltevreden, Tjitjeureub, Bandoeng, Tasikmalaja, Soerabaja; Sumatra: Bireuen, Lokop; Borneo: Boelongan; Timor: Koepang; Nieuw-Guinea: Toerai; (1923) Java: Soemedang, Gombong, Petemon, Magelang, Malang; Sumatra: Moeara Tebo, Padang, Takengon, Lho Seumawe; Ceram: Piroe.

whitmorei (= *Leucomyia plegepennis*, Theo.): Java: Weltevreden.

(subgenus: *Culiciomyia*):

fragilis ?: Borneo: Bandjarmassin, Tanah Grogot, Samarinda, Boelongan; Ceram: Piroe.

pullus: Java: Weltevreden; (1923) Sumatra: Moeara Tebo.

spathifurca: Java: Weltevreden; Sumatra: Lokop (? ♀);

(1923) Alor: Kalabahi; Sumatra: Lho Seumawa (? ♀).

(subgenus: *Lophoceratomyia*):

cinctellus (= *L. taeniata*, Leic.): Borneo: Tanah Grogot (1923) Java: Weltevreden.

fraudator (= *L. fraudatrix*): Java: Weltevreden.

rubithoracis: Java: Weltevreden.

sp.? (♀ 's): Borneo: Boelongan; (1923) Java: Weltevreden; Sumatra: Moeara Tebo.

aga, J.
1923.

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van den Schrijver

Filariasis-onderzoek over dag,

DOOR

Dr. J. HAGA.

*(Mededeeling uit het Centraal Militair
Geneeskundig Laboratorium).*

* * *

*Overgedrukt uit het
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Afl. 6 Deel 63.*

Javasche Boekhandel & Drukkerij.
BATAVIA 1923

Filariasis-onderzoek over dag,

DOOR

Dr. J. HAGA. *)

(Mededeeling uit het Centraal Militair Geneeskundig Laboratorium).

Terecht zeide Prof. KUENEN in de Februari-vergadering van 1921 van de Afdeeling Batavia der Vereeniging tot Bevordering der Geneeskundige Wetenschappen in Nederlandsch-Indië (1), dat het onderzoek naar filariadragers ook over dag kan geschieden, waardoor het onderzoek veel vereenvoudigd wordt, daar toch vooral massa-onderzoekingen onder de bevolking 's avonds moeilijkheden opleveren. Hij geeft tevens een methode aan, die, voor zoover mij bekend, de eenvoudigste en gemakkelijkst uitvoerbare van alle daarvóór beschreven methoden is.

Men vangt daartoe 10 tot 20 druppels bloed op in physiologische zoutsolutie, waaraan een paar druppels van een 1%-saponine-oplossing (al dan niet met 5% carbol) zijn toegevoegd. De saponine haemolyseert de erythrocyten, en de leukocyten plus mikrofilariae kunnen worden afgecentrifugeerd.

KUENEN geeft ook aan, dat men op deze wijze meer filariae vindt dan met de dikke-druppel-methode.

Werkelijk zou het m.i. onnoodig zijn een andere methode aan te geven, indien aan deze niet twee bezwaren kleefden. Het eerste bezwaar is, dat bij niet snel werken, d.w.z. bij een wachten van langer dan 5-10 minuten na de bloedafname, re in de buis stolling begint op te treden, en men dan genoodzaakt is te wachten, tot deze afgeloopen is, waarna men het coagulum kan losmaken, terwijl de mikrofilariën dan in de vloeistof aanwezig zijn.

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*) Gedeeltelijk naar een voordracht, gehouden voor de Afd. Batavia op 19 Juli 1923. De noten in den tekst verwijzen naar de literatuur.

Een tweede bezwaar echter, dat voor de practische uitvoering van deze methode door de officieren van gezondheid veel grooter is, bestaat in het moeten gebruiken van saponine. Deze stof toch komt in de Prijslijst van het Departement van Oorlog, VIde Afd. Militair Geneeskundige Dienst (1918 en 1921) slechts voor op de lijst van die artikelen, welke *uitsluitend* bestemd zijn voor het Scheikundig Laboratorium en het Centraal Militair Geneeskundig Laboratorium te Weltevreden. Daardoor krijgt de buiten die laboratoria werkende officier van gezondheid op aanvraag dat artikel niet.

Toen ik 26 October 1921 te Singkel (Atjeh, S. W. K.) den dienst van den off. v. gez. I. kl. A. J. BARTO overnam, vertelde deze mij, dat hij na kennisname van het door Prof. KUENEN aangegevene het filariasis-onderzoek (dat den officieren van gezondheid in Atjeh sinds Juni 1921 door den Inspecteur van den M.G.D. opgedragen is) verrichtte met behulp van een zeer practische wijziging. Hij had nl. gezocht naar een stof, geschikt om de benoodigde saponine te vervangen. Toen hij deze gevonden had, ondervond hij echter last van het reeds door mij genoemde, eerste bezwaar, nl. een intredende stolling, als men de buisjes te lang liet staan. Bij het onderzoek van telkens slechts vier proefpersonen werd geen last ondervonden. Hij had nog overwogen, om door toevoeging van hirudine (te verkrijgen uit plaatselijk te vangen bloedzuigers) de stolling te voorkomen, maar door de overplaatsing is deze proef achterwege gebleven. Ik sloeg hem echter voor, om ditzelfde gemakkelijker te bereiken, citras natricus toe te voegen (oorspronkelijk in de sterkte van 0,2°/₀, later door mij op 0,3°/₀ verhoogd), welke methode toen nog door ons gezamentlijk is beproefd, uitstekend slaagde en sinds dien ongewijzigd werd toegepast 1).

Dien geschikten saponine-vervanger dan vond BARTO ten slotte in de z.g. zeepvruchten, die elke Inlander kent, daar hij er bij gebrek aan zeep zijn kleeren mee wascht, en die bij den toekang sepoe (toekang mas) ook te krijgen zijn, daar deze zijn goud- en zilverwerk er mee oppoetst. Van deze zeepvrucht kenden we slechts de namen *boea lerak* of *boea tandikir*, onder welken naam ze ook te Padang bekend is. De Atjeh'sche naam is ons onbekend gebleven. Den zeepvruchtboom, den *pohon lerak*, die veel op den *Tamarinde-boom* lijkt, vond BARTO in de buurt van Roendeng (Simpang Kiri, onderdistrict van Singkel).

Meer gegevens hieromtrent kon ik te Singkel ook nie verzamelen, echter kreeg ik waardevolle gegevens van den Dir. Apotheker der 2de kl. Dr. J. VERMANDE, den Directeur van het Scheikundig Laboratorium alhier.

De *boea lerak* is afkomstig van den z.g. zeepnootboom, behoorende tot de familie der *Sapindaceae*, nl. den *Sapindus Rarak*. DE CLERCQ en GRESHOFF (2) geven voor de vrucht, resp. den boom verschillende inheemsche namen op, welke ik hier voor het gemak laat volgen:- Lerek of Bhoengkana lerek (Madoer.); Boewah saboen (vulg. Mal.); Derog (Lamp.); Kelerek (O. Jav.); Lerak 2) (Jav., Mal.); Pohon

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1) Het voorstel echter om citras natricus toe te voegen was niet zoo gemakkelijk uit te voeren als dit lijkt. De moeilijkheid was nl., dat in 1921 ook citras natricus niet in de Militaire Apotheken, noch toen in de Prijslijst van het D v.O. voorkwam. We konden het echter bereiden, daar acidum citricum en bicarbonas natricus in voldoende hoeveelheid aanwezig waren. Door bij een geconcentreerde oplossing van bicarbonas natricus voorzichtig (om een te sterke koolzuurontwikkeling te voorkomen) acidum citricum in overmaat toe te voegen, en de vloeistof daarna in open schalen langzaam aan de lucht te laten verdampen, kregen we het citras natricus in kristallen, welke later door oplossen in water, filtreeren en wederom langzaam in platte schalen aan de lucht te laten verdampen geheel zuiver konden worden verkregen. Sinds 1 Januari 1923 komt het citras natricus in genoemde Prijslijst voor en kan door iederen officier van gezondheid aangevraagd worden.

2) Ook Phrynium griffithii en P. parviflorum Roxb.

saboen (Amb., Mal.); Rerak (Mal.); Rerek (Balin., Soend.); Tampar (of Tapar) boera (Bat.); Tandikije (Minangk.).

Behalve voor het wasschen der kleederen wordt van de vrucht nog gebruik gemaakt om er „jachtwater" van te maken (tot zuivering van hoofdhaar en dooden van ander ongedierte), terwijl ze met succes ook tegen schurft aangewend wordt (3). Wat hierover geschreven staat in het *Geneesk. Tijdschr. v. Ned.-Indië*, Dl. IV, 1855 (4), is aardig genoeg om geheel hieronder te laten volgen:

„In de tweede helft van het jaar 1852 werd de aandacht der redaktie, door den particulieren geneesheer te Probolingo K BUWALDA, verzocht, op de goede uitkomsten verkregen door de aanwending van de vrucht der Sapindus barak tegen scabies en wel op de volgende wijze. Hij liet de vruchten voor het gebruik een uur in water weken en alsdan ontdaan van hun pitten tusschen de handen van eenen helper heen en weer wrijven tot er schuim te voorschijn kwam; met dit schuim werden de schurftigen hetzij door middel van een wollen lap, hetzij met de vlakke hand gedurende ruim een half uur ingewreven en daarna met koud water afgewasschen; voor elke wrijving waren acht à tien vruchten noodig.

„Van de 18 lijders genazen er 10 in vier dagen, de overigen allen binnen de zeven dagen.

„Sedert zijn met afwisselend gevolg in den geest van het vorenstaande proeven genomen.

„De redaktie hierop de opmerkzaamheid van H.H. Medici vestigende, teekent daarbij aan, dat Sapindus barak door den Inlander genaamd *Boea rarak*, *Lerak*, behoort tot het geslacht Sapindus, welke naam is afgeleid van de eigenschap der schors van de hiertoe behoorende planten, om het linnen van vuil te ontdoen even als zeep. Het geslacht Sapindus behoort tot de Octandria monogynia van LINN. en heeft zijn naam gegeven aan de natuurlijke familie of orde Sapindaceae J., die ENDLICHER brengt tot zijne 53ste klasse, die der acera."

In het buitenhulsel der rondachtige vrucht (circa 25 mm. middellijn) zit een saponine (sapotoxine), dat aangeduid wordt als *Sapindus-saponine* of *Saponine Rarak*, en voor 13,5°/₀ van het gewicht van dat hulsel uitmaakt (5). Volgens KOBERT (6) geeft een verdunning in physiologische NaCl-oplossing van dit sapotoxine van 1:13000 tot 1:15000 nog een volledige haemolyse van gedefibrineerd runderbloed, dat met physiol. NaCl-opl. honderdvoudig verdund is.

Ik heb nu van meerdere vruchten het hulsel gewogen en kwam daarbij op zeer verschillende gewichten. Zoo woog bijv. het hulsel van één vrucht 2,750 gram. Van vier goed droge vruchten wogen de hulsels samen 13,040 gr., elk hulsel dus gemiddeld 3,260 gr. Van 4 andere, eveneens goed droge vruchten wogen de hulsels samen 15,220 gr., dus gemiddeld elk hulsel 3,805 gr. Er is dus wel een groot verschil in gewicht bij verschillende vruchten te constateeren, hetgeen dus natuurlijk voor de kracht der haemolytische werking van de van één vrucht afkomstige hoeveelheid vloeistof niet onverschillig kan zijn.

Na deze uitweiding moge dan de *methode van werken*, zooals die na de toevoeging van citras natricus gebezigd werd, hier volgen:

Het saponine-houdend buitenwandje wordt van de rondachtige vrucht afgenomen en in een mortier met wat 0,9°/₀ NaCl-oplossing fijn gewreven (gemakkelijker gaat dit na toevoeging van een weinig steriel zand), daarna met wat NaCl-oplossing aangelengd (tot circa 100 cc.), gefiltreerd door een papieren filter, en het filtraat met de NaCl-oplossing aangevuld tot 0,9 L. vloeistof. Hieraan voegt men toe 0,1 L. eener 3°/₀-oplossing van citras natricus, zoodat ontstaat 1 L vloeistof, welke 0,81°/₀ NaCl, saponine en 0,3°/₀ citras natricus bevat. Van deze vloeistof brengt men 9 cc. in een centrifugeerbuisje, en laat hierin 10 of 20 druppels bloed (circa $^1/_2$ tot 1 cc., beter de grootere hoeveelheid van 1 cc.), door venaepunctie verkregen, van de(n) te onderzoeken persoon vloeien, sluit de buis met den duim af, draait haar twee keer om, waarna de haemolyse reeds volledig is. Stolling treedt nu in het geheel niet meer op. Daarna worden de buizen een 2 minuten (bij circa 2000 toeren) gecentrifugeerd, de bovenstaande vloeistof afgeschonken en het sediment in zijn geheel op een objectglas gebracht. Het sediment bestaat alleen uit leucocyten, en bij aanwezigheid van microfilariën ontdekt men deze reeds bij 50-voudige vergrooting gemakkelijk genoeg aan de door de levendige beweging der dieren veroorzaakte opschudding onder de leucocyten. Het onderzoek is daardoor in één minuut reeds beëindigd. Wil men de soort van microfilariën nog nagaan, dan kan men de massa op het objectglas laten opdrogen, daarna dit in gedestilleerd water brengen om het haemoglobine te laten uittrekken, en kan, nadat het praeparaat weer luchtdroog is geworden, het kleuren volgens KIEWIET DE JONGE (of GIEMSA) of, ter verkrijging van blijvende praeparaten, met DELAFIELD's haematoxyline en dan insluiten in canadabalsem.

De resultaten met deze methode verkregen, waarbij wij slechts over dag, van 10 u.v.m. tot circa 4 u.n.m., de personen op mikrofilariën onderzochten, kunnen niet anders dan schitterend genoemd worden. Zoo onderzocht BARTO 159 personen, waarvan bij 43 het onderzoek positief uitviel. Ik kon slechts 25 personen onderzoeken met een positief resultaat bij 5, zoodat op een gezamentlijk geheel van *184* er *48 filariadragers*, gemiddeld *26*°/₀ konden worden aangetoond. Van deze 184 personen vertoonde slechts één gevolgen der infectie, nl. een elephantiasis van een been, maar bij dezen man viel het onderzoek *negatief* uit. De overige 183 waren geheel willekeurig gekozen.

Over de ongelijkmatige wijze van voorkomen der filariasis kan men een goeden indruk verkrijgen, wanneer men het resultaat zou rangschikken naar de onderzochte kampongs. De gegevens hiervan heb ik helaas momenteel niet tot mijn beschikking, maar wel de samenvatting naar de districten

Beneden-Singkel, *Simpang-Kiri* en *Simpang-Kanan*, en het zelfbesturend Landschap *Troemon*. Deze in een lijst gerangschikt geven het volgende te zien:

Filaria-gegevens der Onderafdeeling Singkel van Juni '21 t/m April '22.

	Beneden-Singkel.	Simp. Kiri.	Simp. Kanan.	Troe-mon.	Elders.	Totaal.
door BARTO onderzocht op mikrofilariën:	16	86	45	10	2	159
waarvan positief:	1	33	5	3	1	43
door HAGA onderzocht op mikrofilariën:	16	2	5	1	1	25
waarvan positief:	0	2	2	1	0	5
totaal onderzocht:	32	88	50	11	3	184
waarvan positief:	1	35	7	4	1	48
voorloopig pos. %-age:	3,1	39,8	14	36,4	33,3	gem. 26.

De proefpersonen waren allen volwassen mannen. Met opzet, met het oog op de bevolking, werden geen vrouwen in het onderzoek betrokken, om geen tegenwerking gaande te maken. Van den kant der proefpersonen is nooit eenige last ondervonden bij de te ondergane venaepunctie. 16 Personen konden gemakkelijk in een uur onderzocht worden.

Het groote voordeel dezer methode, dat men niet in den laten avond, na 9 uur, met het onderzoek heeft te beginnen, is zonder meer duidelijk. Bij in een ziekenhuis opgenomen patiënten bestaan slechts de bezwaren voor den patiënt en den medicus, bij het onderzoek daarbuiten en vooral op de buitenbezittingen worden de bezwaren vrijwel onoverkomelijk.

Hoe immers gaat het daar toe? Bij een tournée van den medicus worden op een bepaalde plaats in den loop van den morgen of vroeg in den middag de inwoners van verschillende kampongs bijeengeroepen. Dan heeft men immers de mooie gelegenheid voor onderzoek. Men heeft goed licht, geen last van muskieten en men kan de inwoners op zijn gemak ondervragen en onderzoeken. In den naävond is microscopiseeren uitgesloten, de muskieten maken dikwijls een verblijf buiten de klamboe onmogelijk, maar het ergste is, dat de inwoners der andere kampongs dan die, waar men huist, alle verdwenen zijn, en de inwoners van de kampong, waar men verblijf houdt, niet komen willen. Over dag is men bovendien in staat inwoners van kampongs te onderzoeken, waar men zelf nagenoeg niet of hoogst bezwaarlijk komen kan. Ik noem het voorbeeld van een in Singkel gelegen kampong Si Gadja[2], die circa een dag loopen verwijderd ligt van het aan de kust gelegen plaatsje Boeloe-Seuma, dat weder eerst na een dag reizen per prauw en te voet van uit Singkel te bereiken is. In den drogen tijd gaat de tocht van Boeloe-Seuma naar S.G.[2] door moerassige streken en mangrove-vegetatie, in den natten tijd gaat men dikwijls tot aan de borst door het water, moet over bandjirende kali's, waarvan soms nog de bruggetjes weggeslagen zijn.

Hoe wordt en werd tot nu toe in Nederlandsch-Indië het onderzoek voornamelijk verricht? Alle onderzoekingen, die ik kon nagaan, hadden 's avonds plaats (bij één patiënt van den off. v. gez. VAN DER KODDE echter ook over dag). Zoo noem ik o.a. de onderzoekingen van FLU (1918 en 1920), KUIJER (Soemba 1920) en de met name aangeduide onderzoekingen in het „Beknopt overzicht betreffende de filaria-verspreiding in Nederlandsch-Indië" van de officieren van gezondheid BOLHUIS (p. 39), VAN DER KODDE (p. 40) en VAN LUYT (p. 42-44) (7).

In datzelfde overzicht is echter verzuimd er op te wijzen, dat in tegenstelling met de andere onderzoekingen *die te Singkel steeds over dag* geschied zijn, hetgeen de resultaten nog veel interessanter maakt.

Een gedeelte uit het geciteerde jaarverslag van Djambi over 1921 van collega BOLHUIS wil ik even aanhalen, waar deze ook duidelijk mijn bovengenoemde bezwaren, verbonden aan het nachtelijk onderzoek, onderstreept:

„Het onderzoek, dat zal worden voortgezet, gaat dikwijls met moeilijkheden gepaard, vooral door het ongelegen uur, waarop de filarialarven zich voornamelijk in het perifere bloed vertoonen en verder door de zeer gemengde bevolking, die de kampongs om Djambi bewonen. De kampongs, die meest dun bevolkt zijn, liggen over grooten afstand langs de kali uitgestrekt".

Wat voor uitkomsten levert nu BARTO's methode (zooals ik haar maar ter eere van den bedenker noemen zal) op, bij vergelijking met de andere, dikke-druppel-methode, indien men het onderzoek over dag doet? Voor een dikken druppel kan men, evenals bij het malaria-onderzoek, een willekeurigen dikken druppel (door prik in vingertop of oorlel verkregen) nemen, of uitgaan van een geijkte, door middel van een meetpipet afgemeten hoeveelheid bloed Voor een goede vergelijking werd aan deze laatste methode de voorkeur gegeven, en werd de door LOW [(8) p. 205, en (9)] in 1913 aangegeven „standaardhoeveelheid" van 20 cmm. (een pipet van SAHLI's haemoglobinometer) voor de dikke-druppel-praeparaten genomen.

Na mijn komst hier wachtte ik steeds op een gelegenheid om de methode eens te contrôleeren. Eind October 1922 bood zich deze aan. In een door collega VAN LOON gezonden dikke-druppel-praeparaat van den patiënt Sadji (Atjeher) van het Doorgangshuis alhier werd bij het malaria-onderzoek een Microfilaria nocturna gevonden. Na overleg met collega VAN LOON en den vorigen Chef van het Militaire Hospitaal alhier, den Dir. off. v. gez. I. kl. D. H. ARENDS kon deze patiënt eenige dagen voor nader onderzoek op de Blokzaal worden opgenomen.

Dit onderzoek geschiedde 3 November 1922 te zamen met collega J. K. DEN HEIJER. Om BARTO's methode zoo scherp mogelijk op de proef te stellen en tevens zoo zuiver mogelijk te werk te gaan, werd als uur van onderzoek het voor een onderzoek op Microfilaria nocturna het als zoo ongunstig mogelijk te boek staande tijdstip van 12 u. v. m. gekozen. Verder werd als volgt gehandeld. 16 Centrifugeerbuizen werden elk gevuld met 9 cc van de bovengenoemde NaCl-saponine-citras-natricus-oplossing. Met een Recordspuit van 10 cc. werd door mij door middel van punctie der vena cubiti van den linker arm 10 cc. bloed opgezogen en in elk van 14 centrifugeerbuizen $^1/_2$ cc. bloed gebracht. Het was nu de bedoeling de resteerende 3 cc. bloed gelijkelijk over de overblijvende 2 centrifugeerbuizen te verdeelen. Één buis, B, kreeg de voor haar bestemde helft, dus 1$^1/_2$ cc. bloed wel, de andere, A, kreeg door optredende stolling nog geen $^1/_2$ cc. bloed, en mag dus feitelijk niet voor het onderzoek meetellen. Ondertusschen was collega den Heijer ook aan het werk getogen en met de meetpipet van 20 cmm. verkreeg hij van het rechter oorlelletje bloed voor 7 praeparaten. Hierbij werd de 20 cmm. bloed per praeparaat over een oppervlak van circa 3 × 2 cm. uitgestreken. Deze praeparaten werden na drogen door den Heijer onthaemoglobiniseerd, gekleurd volgens Kiewiet de Jonge, maar *bleken in het geheel geen microfilariae te bevatten.*

De door mij verkregen 16 centrifugeerbuizen (waarin het bloed door eenmaal omdraaien — niet schudden — van de buis volledig gehaemolyseerd was) wilde ik benutten voor nog een ander onderzoek, nl. om na te gaan hoe lang de microfilariën in die vloeistof in leven bleven. Te Singkel had ik reeds gevonden, dat na een verblijf van 65 min. bij de gewone buitenluchttemperatuur (circa 28°C.) in deze vloeistof de microfilariën niets van hun beweeglijkheid hadden verloren. Hier leerde ik uit Fülleborn's verhandeling (8), dat de microfilariën volgens Mandel in bloed met citras natricus meerdere dagen in leven bleven (F. vermeldt niet, bij welke temperatuur deze proef verliep) Wat echter geschiedde in een oplossing van citras natricus met saponine vond ik niet vermeld. In elk geval wilde ik de temperatuur voor het in leven blijven der microfilariën zoo gunstig mogelijk maken, en daar dit de temperatuur der ijskist is (8), werd het rekje met de 16 buisjes in de ijskist geplaatst. Na verschillende iden werden dan buisjes er uit genomen en op de microfilariën onderzocht.

Het volgende staatje geeft nu een goed overzicht:

Buisje N°.	Na verblijf in ijskist van:	Aantal levende microfilariën:	Aantal onbeweeglijke (doode) microfilariën:	
1	1 uur	2		
2	„	2		
3	5 uur	6		
4	„	1		
5	20 uur	1		
6	„	4		
7	29 uur	4		
8	45 uur	—	8	De microfilariën waren eerst na kleuring (Delafield's haematoxyline) te zien.
9	„	—	1	
10	„	—	5	
11	„	—	1	
12	„	—	0	
13	„	—	1	
14	„	—	3	
(A)	„	—	(0)	
(B)	„	—	4	
	Totaal:	20	23	
	Te zamen:	43		
	in de eerste 14 buizen te zamen:	39		

Rekenen we dus alleen de microfilariën van de eerste 14 buizen, die een gelijke hoeveelheid ($^1/_2$ cc.) bloed gekregen hebben, dan tellen we toch 39, d.i. bijna een gemiddelde van 3 mf. per buis.

Tegenover de dikke-druppel-methode geeft dus deze methode afdoende bewijzen van haar superioriteit. Feitelijk is dit ook niet verwonderlijk, gezien de veel grootere hoeveelheid bloed, die daarbij gebruikt werd (in plaats van 1 × of 4 × 20 cmm. ineens 500 cmm.), maar toch stemt dit tot groote voldoening. Immers, waar de eene methode steeds een negatief resultaat opleverde, gaf deze bij een 14-voudige beproeving in 13 maal een positief resultaat. Bovendien, zooals reeds gezegd, heb ik de methode scherp op de proef willen stellen, daar ik in de praktijk nagenoeg steeds 1 cc. bloed nam. Het is dan ook mijn vaste overtuiging, dat het bij een voortgezet onderzoek blijken zal, dat men ook over dag dezelfde positieve resultaten als 's avonds krijgen zal, als men voor deze onderzoekingen een 10, liefst 20 druppels bloed (door venaepunctie verkregen circa $^1/_2$-1 cc.) gebruikt.

Ook leert deze proef, dat de microfilariën in elk geval gedurende een verblijf van minstens 29 uur in genoemde oplossing en bij ijskisttemperatuur in leven blijven.

Proeven, genomen met de oplossingen van de boea lerak:

Voor deze proeven ben ik uitgegaan van de hulzen van 5 vruchten, te zamen wegende 17,970 gr. (d.i. per vruchthuls 2,994 of vrijwel 3 gr.) Deze 5 hulzen werden elk afzonderlijk in een mortier met *aq. dest.* zeer fijn gewreven en dit extract door een papierfilter gezeefd, en met *aq. dest.* aangevuld, zóó dat de 5 vruchten precies 500 cc. extract opleverden. Daar deze oplossing dus 10 maal zoo sterk is, als voor de methode vereischt wordt, zij hier even vermeld, dat voor de latere proeven alle oplossingen, van dit extract afkomstig, 10 maal verdund werden door op 1 deel extract 9 dl. physiologische (0,9$^0/_0$-) NaCl-oplossing toe te voegen.

Dit extract zullen we de standaard-saponine-oplossing, S.S., noemen, en zij werd benut voor de volgende 5 proefoplossingen:

I. 135 cc. S.S. werden vermengd met 15 cc. 5$^0/_0$ carbol (d.i. 9 dl: 1 dl.), resultaat 150 cc. carbol-saponine-oplossing, C.S.

II. 100 cc. S.S. werden in een flesch in de autoclaaf gedurende één uur bij 120° C. verhit: A.S.

III. in elk van 2 steriele reageerbuizen werd gebracht 10 cc. S.S, en beide buizen werden op 3 achtereenvolgende dagen in een waterbad bij 56° C. gedurende één uur verhit: W.S.

IV. 50 cc. S.S. werden in een kolf op asbestgaas gedurende 20 minuten gekookt, K S.

V. Nadat de oplossing W.S. (ziet III) gereed was, werd een gedeelte der S.S. niet chemisch of thermisch behandeld, doch slechts *10 × verdund,* door bij 10 cc. S.S. te voegen 90 cc. 0,9% NaCl-oplossing, waardoor we dus krijgen 100 cc. 0,81% NaCl-saponine-oplossing. Deze zullen we de proefsaponine-oplossing P.S. noemen.

De met deze verschillende oplossingen ingestelde proeven beoogden na te gaan, hoe de haemolytische kracht beïnvloed werd door de thermische of chemische sterilisatie, en hoe groot ze feitelijk wel is, want aan de opgaven van KOBERT (ziet boven) had ik voor deze praktische toepassing niets. Tevens wilde ik nagaan, welke grenzen voor de te nemen bloedhoeveelheid te stellen waren. Te Singkel had ik reeds ondervonden, dat men aan de 9 cc. saponine-citras-natricus-oplossing maar een beperkte hoeveelheid bloed kan toevoegen, daar ik eenmaal, toen ik ongeveer 2 cc. bloed had laten toevloeien en die buis toevallig het laatst onderzocht, het geheele gehaemolyseerde mengsel gelatineus gestold vond.

Voor de proeven werd gebruik gemaakt van normaal menschenbloed en van erythrocyten van menschenbloed en schapenbloed afkomstig. Ter verkrijging der erythrocyten werd het bloed, door venaepunctie verkregen, opgevangen in 2% citras-natricusoplossing, in verhouding van 4 dl. bloed op 1 dl. citr.-natr.-opl. Na wat schudden wordt deze hoeveelheid aangelengd tot een 3-voudig volumen met 0,9%

NaCl-opl., dan 10 min. matig hard gecentrifugeerd, de bovenste vloeistof afgezogen, weer aangevuld met NaCl-opl. enz. en de erythrocyten op deze manier net zoo lang gewasschen, tot de hiervan afkomstige keukenzoutoplossing met de azijnzuur-ferrocyaankalium-proef geen eiwitreactie meer geeft. Dan worden deze erythrocyten met 0,9°/₀ NaCl aangelengd tot de oorspronkelijke hoeveelheid bloed (zonder citras-natricus-opl.), en dit is de 100°/₀-erythrocyten-emulsie. Een 20°/₀-schapenerythrocyten-emulsie had een haemoglobine-gehalte volgens Tallquist van 30-40.

Bij deze proeven wordt de *haemolyse aangeduid* met:

c. = compleet; b.c. = bijna compleet; b.c.-c. = bijna compleet tot compleet; zw.r. = zwakke remming; d.r. = duidelijke remming; st.r. = sterke remming; t.r. = totale remming.

Toen alle oplossingen gereed waren, dus 4 dagen na het bereiden van het extract, werd met de proeven begonnen.

A. Deze proef moest dienen om na te gaan, of de toevoeging van carbol ook een ongunstigen invloed had op de haemolytische werking der saponine-oplossing. De carbolsaponine-oplossing C.S. werd ook verdund door toevoeging van 9 deelen NaCl-opl. Daar echter C.S. slechts 0,9 der hoeveelheid saponine bevat als de niet met carbol vermengde oplossing, werd in de proeven met C.S. steeds slechts 0,9 van de hoeveelheid bloed (of erythrocyten) genomen als in de proeven met de P.S.

Het bleek nu, dat men bij de meer geconcentreerde erythrocyten-oplossingen, waarin na de eindverdunning meer dan 20°/₀ erythrocyten was, een duidelijk verschil ten nadeele der carboloplossing kon waarnemen (ziet de buizen 1a en 1b, en 2a en 2b), maar bij een lager procentage, en *reeds van af een eindverdunning van 8°/₀* (ziet 3a en 3b) was het *verschil nauwelijks of in het geheel niet meer waarneembaar* (ziet onderstaande tabel).

I. Proef met schapenbloederythrocyten en de opl. P.S. en C.S.

Buisje N°.	Hoeveelheid en concentratie der erythroc.	Hoeveelheid P. S.	Hoeveelheid C. S. (10 × verdund)	%age eindverdunning der erythr.	Haemolyse bij P. S.	Haemolyse bij C. S.
1a	0,1 cc. 80%	0,06 cc.	—	50%	b. c.	
b	0,09 „	—	0,06 cc.	„	—	st. r.
2a	0,3 cc. 40%	0,3 cc.	—	20%	b. c — c.	
b	0,27 „	—	0,3 cc.	„	—	d. r.
3a	0,8 cc. 16%	0,8 cc.	—	8%	c.	
b	0,72 „	—	0,8 cc.	„	—	b. c.
4a	0,4 cc. 12%	0,4 cc.		6%	c.	
b	0,36 „	—	0,4 cc.	„	—	b. c.
5a	0,1 cc. 8%	0,1 cc.	—	4%	c.	
b	0,09 „		0,1 cc.	„	—	c.
6a	1 cc. 2%	1 cc.	—	1%	c.	
b	0,9 „	—	1 cc.	„	—	c.

B. De tweede proef toont aan, hoe sterk men de saponineoplossingen I en V, d. z. de C.S. en de P.S.-opl., *nadat de eerste natuurlijk evenals in de voorgaande proef 10-voudig verdund was*, nog kan verdunnen in een oplossing, waarin steeds evenveel schapenbloederythrocyten aanwezig waren, zonder dat de haemolytische werking wordt opgeheven.

Hierbij werd uitgegaan van een 8% schapenbloed-erythrocyten-emulsie, die door toevoeging van een geleidelijk afnemende hoeveelheid saponine-oplossing en daarmede evenredig stijgende hoeveelheid NaCl-oplossing steeds tot het dubbele volumen gebracht werd, zoodat de eindverdunning in alle buizen een 4%-erythrocyten-emulsie bedroeg. Het bleek daarbij (ziet onderstaande tabel), dat zelfs na een 5-voudige verdunning (buizen 5a en 5b) de C.S.- en P.S.-oplossingen nog in staat waren in een gelijk volumen 8% schapenbloed-erythrocyten een complete haemolyse te veroorzaken.

II. Proef met 8°/₀ sch. bl. erythr. en de saponine-oplossingen P. S. en C. S.

Buisje N°.	Hoeveelheid erythrocyten	Hoeveelheid P. S.	Hoeveelheid C. S. (10 × verdund)	0,9 % NaCl oplossing	Haemolyse bij P. S.	Haemolyse bij C. S.
1a	0,1 cc.	0,1 cc.	—	—	c.	
b	0,09 cc.	—	0,1 cc.	—	—	c.
2a	0,2 cc.	0,1 cc.	—	0,1 cc.	c.	
b	0,18 cc		0,1 cc.	0,08 cc.	—	c.
3a	0,2 cc.	0,07 cc.	—	0,13 cc.	c.	
b	0,18 cc.	—	0,07 cc,	0,11 cc.	—	c.
4a	0,2 cc.	0,05 cc.	—	0,15 cc	c.	
b	0,18 cc.	—	0,05 cc.	0,13 cc.	—	c
5a	0,2 cc.	0,04 cc.	—	0,16 cc.	c.	
b	0,18 cc.	—	0,04 cc.	0,14 cc.	c.	c.

C De nu volgende proef, genomen met de saponine-oplossingen II en III, d.z. de A.S. en W.S.-oplossingen, eveneens eerst 10-voudig verdund, toonde aan, dat deze oplossingen bij niet te sterke verdunning onder dezelfde omstandigheden als bij de vorige proef in een gelijk volumen 8°/₀ schapenbloed-erythrocyten een complete haemolyse veroorzaakten. maar reeds bij een 3-voudige en nog erger bij een 4-voudige verdunning (ziet onderstaande tabel buizen 2 en 3a en 3b) een duidelijke remming vertoonden. Dit bewijst dus, dat de sterilisatie door verhitting op de haemolyseerende werking een veel ongunstigeren invloed uitoefent dan de sterilisatie door carbol.

STAAT.

III. Proef met 8°/₀-sch. erythr. en de saponine-oplossingen A.S. en W.S.

Buisje N°.	Hoeveelheid erythrocyten	Hoeveelheid A.S. (10 X verdund)	Hoeveelheid W.S. (10 X verdund)	0,9 °/₀ NaCl oplossing	Haemolyse bij A.S.	Haemolyse bij W.S.
1a	0,2 cc.	0,1 cc.	—	0,1 cc.	c.	c.
b	0,2 „	—	0,1 cc.	0,1 „	—	
2	0,2 „	0,07 cc.	—	0,13 „	d. r.	
3a	0,2 „	0,05 „	—	0,15 „	st. r.	
b	0,2 „	—	0,05 cc.	0,15 „	—	d.-st. r.
4	0,2 „	0,04 cc.	—	0,16 „	st. r.	
5a	0,2 „	0,02 „	—	0,18 „	t. r.	
b	0,2 „	—	0,02 cc.	0,18 „	—	t. r.

D. Nadat de vorige proef geleerd had, dat verhitting dus een ongunstigen invloed uitoefende op de haemolytische werking der saponine-oplossing in sterke verdunningen, werd slechts een vergelijkende proef ingesteld betreffende de haemolytische werking der oplossingen IV en I, d. z. K. S. en C. S, wederom na 10-voudige verdunning der beide oplossingen. Daartoe werden gelijke volumina van deze kooksaponine-oplossing, resp. carbolsaponine-oplossing en schapenbloed-erythrocyten in afdalende concentratie van 20°/₀ tot 2°/₀ vermengd, zoodat de eindverdunningen 10°/₀ tot 1°/₀ erythrocyten bevatten. Hierbij bleek, dat er geen verschil tusschen beide saponine-oplossingen aan te toonen was, want in alle buizen onstond complete haemolyse.

E. Ten slotte werd nagegaan, hoe sterk haemolyseerend de saponine-oplossingen I en V, d. z. de C. S. en P. S opl., werkten op menschenbloed, resp. de erythrocyten daarvan. Daartoe werd bij steller dezes venaepunctie verricht en bloed op 2 manieren opgevangen, nl.:

1. in 2°/₀ citras-natricus-oplossing, nl. 4 dl. bloed in 1 dl. citr.-natr.-opl.: dit moest dienen om de haemolytische

werking te kunnen nagaan op een erythrocyten-emulsie.

2. in de P. S.-opl., waaraan op 100 cc. 0,3 gr. citras natricus was toegevoegd. Dit is geheel gelijk aan de methode van werken in de praktijk en diende alleen om vast te stellen, hoeveel bloed men veilig aan 9 cc. dezer saponine-citras-natricus-NaCl-oplossing kan toevoegen zonder hinderlijke verschijnselen te ondervinden.

Nadat van het op de 1ste manier opgevangen bloed de 100°/₀-erythrocyten-emulsie verkregen was (die volgens Tallquist een haemoglobine-gehalte aanwees van 80, terwijl de 20°/₀ emulsie ervan volgens T. tusschen 30 en 40 aangaf; het kort daarna verrichte onderzoek van het haemoglobinegehalte volgens **Sahli** van het gewone bloed gaf aan 76), werden hiermede de beide volgende proeven ingesteld:

a. 9 buizen werden gevuld met de P. S.-oplossing en 9 andere met 10-voudig verdunde C. S.-oplossing, met geleidelijk steeds met 1 cc. stijgende hoeveelheden, dus van 1, 2, 3, 4, enz. tot 9 cc. In elke buis der P. S.-oplossing kwam nu 1 cc. 100°/₀ erythrocyten-emulsie, en in elke buis der C. S.-oplossing 0,9 cc. van dezelfde emulsie (ziet de redenen hiertoe onder A.).

Het bleek toen, dat 6 cc. van beide saponine-oplossingen in staat waren de onderling overeenkomstige hoeveelheid 100°/₀ erythrocyten-emulsie volledig te haemolyseeren, en dat 5 cc. nog een bijna complete haemolyse gaven. Waar echter 2 cc. P. S. nog in 1 cc. 100°/₀ erythrocyten een bijna complete haemolyse gaf, was in de overeenkomstige buis met 2 cc. C.S. een zwakke remming aanwezig, zoodat bleek, dat de C. S toch iets minder sterk haemolytisch werkte.

b. 7 buizen werden gevuld met afdalende hoeveelheden P. S.-oplossing en aan elke buis werd toegevoegd 1 cc. 10°/₀ menschenbloed-erythrocyten. Het resultaat is in onderstaande tabel gemakkelijk af te lezen.

STAAT.

IV. Proef met 10 $^{0}/_{0}$ menschenbloed-erythrocyten-emulsie en P. S.

Buisje No.	Hoeveelheid erythrocyten	Hoeveelheid P. S.	Haemolyse
1	1 cc.	0,5 cc.	c.
2	id.	0,25 „	b. c.—c.
3	id.	0,15 „	zw. r.
4	id.	0,1 „	d. r.
5	id.	0,05 „	st. r.
6	id.	0,025 „	t. r.
7	id.	0,01 „	t. r.

Bij het op de 2de manier (zooals gezegd dus gelijk aan de methode in de praktijk) opvangen van het bloed direct in het saponine-citras-natricus-NaCl-mengsel bleek, dat de grootste hoeveelheid bloed, die men daartoe zonder hinder nemen kan, $1^1/_2$ cc. is. In 9 cc. opl. gaf $1^1/_2$ cc. bloed nog een complete haemolyse en in de buis trad na 20 min. en ook na 21 uur geen stolling op. Bij toevoeging van 2 cc. bloed aan 9 cc. opl. ontstond een bijna complete haemolyse (hetgeen overeenkomt met de proeven boven onder *a*, p. 881 vermeld), maar na 20 min. begon een gelatineuze stolling in de heele buis op te treden, na 2 uur nam het stolsel 4/5 van de hoogte van de vloeistofkolom in en na 21 uur nog ongeveer 1/10 er van.

F. Ten slotte werden door Dr. M. ERBER in de bacteriologische afdeeling van het C.M.G.L. culturen aangelegd van de saponine-oplossingen, welke door toevoeging van carbol (C.S., opl. I) of door verhitten op het waterbad bij 56° C. gedurende één uur op 3 achtereenvolgende dagen (W.S., opl. III) steriel moesten zijn geworden, op den 5^{den} dag nadat deze oplossingen gereed waren gekomen. *Alle culturen bleven steriel.*

Conclusies der proeven:

1. Men kan dus veilig aan 9 cc. saponine-citras natricus-NaCl-opl. 1 cc. bloed van den te onderzoeken persoon

toevoegen, waardoor nooit stolling en steeds complete haemolyse optreedt;

2. de carbol-saponine-oplossing mag in haemolytische kracht met de onbehandelde saponine-oplossing vrij wel op dezelfde lijn gesteld worden, terwijl de op de andere, meer gecompliceerde wijzen gesteriliseerde saponine-oplossingen iets minder voldoen.

Waar het mij niet gemakkelijk mogelijk is, deze methode aan meer materiaal te toetsen, hoop ik, dat die medici, welke daartoe meer gelegenheid hebben, deze methode eens nader zullen beproeven. De buitengewone gemakkelijkheid, de betrouwbaarheid en de geheel te verwaarloozen kosten der methode (12 zeepvruchten kosten hier te Weltevreden slechts een dubbeltje en zijn voldoende voor een 1300 onderzoekingen!) geven haar daarop volkomen recht.

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aga J.
1924.

22

van den Schrijver

Een geval van infectie met Opisthorchis felineus bij den mensch,

DOOR

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(Centraal Militair Geneeskundig Laboratorium).

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Een geval van infectie met Opisthorchis felineus bij den mensch,

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Op 3 Juli werd bij het routine-onderzoek der faeces van een Menadonees, Infanterist 1e klasse, Stb. N°. 90405, een ei gevonden, dat door zijn buitengewone zeldzaamheid afzonderlijke vermelding verdient.

In de dunbrijige, met bloederig slijm vermengde ontlasting werd gevonden:

Entamoeba histolytica: groote vegetatieve (histolytische) vormen: +, cysten: +, Ascaris-eieren: +, Trichocephalus-eieren: +, en bovendien in de 11 dikke eosine-praeparaten, voor het onderzoek op wormeieren, nog 2 eieren, die door hunne afwijkende grootte en bouw dadelijk opvielen. Ze waren peervorming met een breede en een smalle pool, bezaten een vrij dikke schaal met een dubbele contour, vertoonden aan de spitse pool een duidelijk deksel, en waren 27 × 14 mikra groot.

De buitenomtrek van dit deksel nu bleek zelfs bij zeer sterke vergrooting (1/12″, oc. 4 Zeiss) zonder verdikkingen of andere uitsteeksels aan het grensvlak, in den omtrek van de schaal van het ei als het ware door te loopen. Het deksel was niet hoog.

In het inwendige van het ei was een lichte korreling waar te nemen.

Wegens de bovengenoemde kenmerken werd de diagnose (waarbij Clonorchis sinensis natuurlijk in aanmerking genomen werd) gesteld op *Opisthorchis felineus* [1]).

Den volgenden dag werd nog een ei van dezelfde afmetingen gevonden, maar deze keer bevatte het een fraai miracidium met duidelijke wimperharen.

Daar deze patient voor zijn amoebendysenterie geruimen tijd binnen lag, zijn de faeces nog op 24 verschillende dagen speciaal op wormeieren (10-26 praeparaten per keer) onderzocht, maar de eieren zijn niet meer gevonden.

Behalve nu ook de bovenbeschreven, van het clonorchis-ei afwijkende kenmerken, pleit ook de landaard van den patiënt tegen een infectie met Clonorchis sinensis.

Patiënt is nl. een Menadonees (geboren 1888), die tot 1908 te Menado (Celebes) gewoond heeft, daarna als soldaat naar Java is gegaan en sinds dien nooit naar andere gedeelten van de buitenbezittingen, behalve Menado, of buiten Nederlandsch-Indië geweest is. Bij den Inlander is tot nu toe nog geen infectie met Clonorchis sinensis vastgesteld.

Wegens het merkwaardige geval mogen de anamnese en ziekte-geschiedenis kort vermeld worden:

L........w.: Infanterist 1de klasse, Stb. N°. 90405. Geboren 1888 te Menado (ouders Menadoneezen).

Tot 1908 te Menado; in 1908 geëngageerd als soldaat en naar Java; nooit Java verlaten, behalve in de 1ste helft van 1923 met verlof naar Menado. Pas kort ziek zijnde, van zijn 2-maanden verlof teruggekeerd te Weltevreden, werd hij hier onmiddellijk (2 Juli) opgenomen wegens zijn ziekte in het Militaire Hospitaal. De ziekte begon met buikpijn, bloederigen en slijmerigen frequenten dunnen stoelgang (± 10 × daags), zonder koorts.

Vroeger heeft patiënt slechts éénmaal in 1921 te Weltevreden gedurende 2 dagen lichte diarrhoe gehad (4 × daags ontlasting) zonder bijmenging van bloed en slijm, welke diarrhoe na 2 dagen geheel over was en waarvoor patiënt niet in het Hospitaal is opgenomen. Patiënt heeft geen icterus gehad.

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[1]) Voor uitvoerige gegevens betreffende de Clonorchis sinensis en Opisthorchis felineus moge verwezen worden naar het artikel van Dr. J. MEBIUS, „Clonorchiosis hepatis, cirrhosis parasitaria en typische groei van het galgangepitheel", in de *Mededeelingen van den Burg. Geneeskundigen Dienst in Nederlandsch-Indië*, 1920, Deel V en *Geneesk. Tijdschr. van Nederlandsch-Indië*, 1920, Deel 60, pag. 224-294.

Patiënt woonde te Menado in zijn jeugd bij zijn ouders. Deze hielden er geen honden of katten op na, maar hij kwam bij zijn broeder, die eenige honden (geen katten) hield, met wie hij veel speelde.

Patiënt heeft nooit ongekookte visch gegeten, alleen visch, die goed gekookt was. Verder ook vleesch, maar dit nooit rauw.

De 26 dagen van het onderzoek der faeces vielen in den tijd van 3 Juli-20 Augustus 1923. Eind Augustus heeft patiënt, hersteld van zijn amoebiasis, het hospitaal verlaten.

23

van den Schrijver

Tabellen voor determinatie der in Nederlandsch-Oost-Indië voorkomende Anophelinen,

DOOR

Dr. J. HAGA.

Overgedrukt uit het
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Afl. 4 Deel 70, 1930.

G. Kolff & Co.,—Batavia, Weltevreden.

(*Mededeeling uit het Militair Geneeskundig Laboratorium*).

Tabellen voor determinatie der in Nederlandsch-Oost-Indië voorkomende Anophelinen [1]

DOOR

Dr. J. HAGA.

Daar in de laatste jaren veel nieuwe soorten Anophelinen, min of meer verspreid voorkomend, in Nederlandsch-Indië beschreven, en de bestaande determinatie-tabellen der imagines (die van N. H. SWELLENGREBEL van 1921 en E. RODENWALDT van 1924) daardoor geheel onvolledig en dus niet meer bruikbaar zijn geworden, voelde ik me eind 1929 genoopt voor eigen gebruik en voor het onderricht van officieren van gezondheid nieuwe tabellen te maken. Daarin konden ook de door Dr. H. DE ROOK, Officier van Gezondheid 1ste klasse, in Zuid-Nieuw-Guinea (Boven-Digoel) en Ternate nieuw gevonden soorten (A. amictus, bancrofti, papuae en longirostris var. annulatus) nog opgenomen worden, zoodat de tabellen op 't oogenblik *alle* in Nederlandsch-Indië voorkomende soorten behandelen. Daarbij is de nieuwe nomenclatuur ingevoerd. In verband met deze nomenclatuur en de opmerkingen van G. COVELL, volgens wien A. (Nyssorhynchus) jamesi, die in Nederlandsch-Indië voorkomt, dezen naam ten onrechte draagt, terwijl beide subspecies van A. (Nyssorhynchus) fuliginosus volgens dienzelfden schrijver tot één soort, A. philippinensis samengevoegd moeten worden, zijn dus enkele soorten herdoopt. Een opgave der oude en nieuwe benamingen is aan elke tabel toegevoegd.

Hierbij ben ik verplicht mijn dank te betuigen aan de heeren S. L. BRUG, Dr. H. DE ROOK en Prof. Dr. E. WALCH, voor hun hulp op verschillend gebied.

Tot publicatie van deze tabellen ben ik overgegaan niet alleen wegens de bovengenoemde redenen, maar ook, omdat een nieuwe bewerking van het boek van Prof. Dr. N. H. SWELLENGREBEL, „*De Anophelinen van Nederlandsch-Oost-Indië*,” vermoedelijk nog geruimen tijd op zich zal laten wachten, waardoor veel onderzoekers, vooral in het Oosten van onzen Archipel, de daar gevonden muskieten niet kunnen determineeren.

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[1] Bij de redactie ontvangen 16 Januari 1930.

I. Tabel voor determinatie der wijfjes-Anophelinen.

1 a Abdomen dorsaal van 2de t/m 8ste segment dicht bezet met breede goudgele schubben, en ventraal op 2de t/m 8ste segment iets minder dicht bezet (naar de bases der segmenten toe) met breede witte èn gele schubben; proboscis geheel donker; de vleugelcosta met zes groote donkere vlekken, de 1ste long. met 14—18 kleine zwarte vlekken, geen groote vlekken; palpen met een proximaal smallen ring en drie distale breede witte banden; femur, tibia en tars. 1 van alle pooten geel gevlekt, alle tarsalia 1 t/m 4 apicaal geel geband, de tarsalia 2 t/m 4 tevens basaal geband .... **A. amictus.**

b Abdomen dorsaal op 2de t/m 8ste segment apicaal en mediaan verspreide breede gele schubben, op 2de t/m 7de segment bovendien twee laterale apicale bundels van zeer breede bruine schubben, op 4de t/m 8ste segment staan de gele schubben mediaan ook aan de bases der segmenten en strekken zich apicaal tot aan de laterale bundels uit: op de ventrale zijde van den top van het 2de t/m 7de segment mediaal en lateraal drie groepjes breede witte schubben, het mediane groepje vergezeld van een bundel zeer breede bruine schubben; de vleugelcosta basaal tot aan de inmonding der subcosta met eenige, meest vier, zeer kleine lichte plekken, en distaal, nl. bij de inmonding der subcosta en nabij den top, twee breede, lichte plekken; palpen proximaal met twee smalle, witte ringen en een distalen breeden, het toplid innemenden witten band; femur, tibia en tars. 1 van alle pooten geelgrijs, niet geel gevlekt; van den achterpoot zijn de apicale helft van tars. 1 en de tarsalia 3 t/m 5 geheel wit .... **A. errabundus.**

c Abdomen niet als onder a en b beschreven .... 2.

2 a Vleugel ongevlekt .... 3.

b Vleugel gevlekt .... 7.

3 a De stam der 4de en de boventak der 5de long. zijn gegolfd 4.

b Niet aldus .... 5.

4 a Lengte der zwarte palpen meer dan de helft (tot circa 2/3) van de lengte van die van proboscis; proboscis belangrijk korter dan de voorfemur; eerste vorkcel zeer klein, nl. 1/3 van de lengte van den stam der 2de long. (tot aan de voorste dwarsader) .... **A. bironelli.**

b Lengte der palpen is circa 1/4 van die van proboscis; proboscis belangrijk langer dan voorfemur; eerste vorkcel normaal, is nl. even lang als de stam der 2de long. (de ♂'s en ♀'s zijn, ook wat de antennae betreft, precies gelijk, en alleen aan de genitalia te onderscheiden) .... **A. travestitus.**

c Lengte der donkerbruine palpen is circa 2/5 van die van proboscis; proboscis belangrijk korter dan voorfemur (nl. 5:7); eerste vorkcel bestaat feitelijk niet, daar de ondertak der 2de long. niet in den boventak uitmondt; de stam der 4de long. heeft op de benedenwaartsche uitbochting, welke gelegen is halverwege top 2de vorkcel en achterste dwarsader, op den top dier bocht een verdikking der ader. .... **A. papuae.**

5a Gele vleugel .... **A. immaculatus.**

b Bruine vleugel .... 6.

6a Lengte circa 3,5 mm; kop met zeer smalle vorkschubben; prothoraxlobben zonder schubbenbundel; middelste dwarsader iets vòòr de voorste gelegen, de achterste dw. a. voor 1,5 maal haar lengte achter de middelste; de 3de long. eindigt meestal in de voorste dw. a., loopt echter soms er voorbij; palpen dun, grijsbruin, ongeband, even lang als proboscis .... **A. aitkenii.**

b Lengte circa 5 mm; kop met breede vorkschubben; prothoraxlobben vooraan met schubbenbundel; middelste dwarsader voor 2/3 van haar lengte *achter* de voorste, en vòòr de achterste dw. a.; de 3de long. loopt achterwaarts voorbij de voorste en middelste dw. a.; palpen meten ongeveer 3/4 van de lengte van de proboscis .... **A. brevipalpis.**

7a Achterfemur vertoont apicaal een zeer breede pluim van zeer lange, spatelvormige witte schubben, basaalwaarts gevolgd door een breeden band van groote elliptische zwarte schuin afstaande schubben. Vleugel met volgende kenmerken: het eind der costa, de top der 1ste long. en de top van den boventak der 2de long. zijn geel beschubd, overigens is de heele vleugel donker; de basis der costa vertoont een lange-, de subcosta ter hoogte van den oorsprong der 2de long. een kleine zwarte vlek; de 2de long. vertoont drie opvallende, reeds met de loupe zeer goed zichtbare zwarte vlekken, nl. 1. aan den oorsprong, 2. ter hoogte van de dwarsaders (overgrijpend op 3de en 4de long.), 3. bij de bifurcatie op beide vorktakken .... **A. annandalei.**

b Achterpoot met breeden lichten tibiotarsalen band .... 8.

c Achterpoot anders dan sub a. en b. .... 9.

8 a Palpen met vier duidelijke witte banden, aan den top der leden, het toplid is apicaal voor de helft tot drie vierde wit (geel); proboscis is normaal lang, even lang als, of heel weinig langer dan, de palpen; de donkere vlekken op de vleugels minder groot; de vlekken op de 1ste long. meer uit elkaar .... **A. leucosphyrus type.**

b De palpen met zeer smalle witte ringen, het toplid alleen wit aan het uiterste einde; proboscis veel langer dan de palpen (deze zijn voor een lengte, gelijk aan de laatste twee palpleden, korter dan de proboscis); de donkere vlekken op de vleugels grooter; de vlekken op de 1ste long. meer in elkaar overloopend .... **A. leucosphyrus var. hackeri.**

9 a Costa (gerekend tot inmonding der 1ste long.) met hoogstens twee duidelijke lichte plekken ....10.

b Costa met meer dan twee lichte plekken ....18.

10 a Palpen geheel donker (soms met iets lichter geelbruine schubben op de grens van 2de—3de en 3de—4de segment) ......11.

b Palpen met drie of vier lichte ringen (soms slechts twee) 15.

11 a Tarsale 5 achterpoot geheel licht (tars. 3 en 4 wit met zwarten band middenin) .... **A. albotaeniatus.**

b Geen der tarsalia geheel licht ....12.

12 a Palpen zeer dik; ventraal op 2de—7de abdominaalsegment een paar witte schubbenbundels aanwezig, èn (of) een groep van 3—20 witte schubben en 3—4 haren op den achterbeugel .... 13.

b Palpen dunner; geen ventrale abdominale schubbenbundels, geen groep van witte schubben op den achterbeugel .... **A. umbrosus.**

13 a Witte spikkels op voorfemur (muskiet van onderen bekijken!); vleugelfranje bij inmonding van 3de long. donker; de groep witte schubben op den achterbeugel vaak geheel afwezig, dikwijls zijn er 1, 2 of 3 schubben, nooit meer ... **A. bancrofti.**

b Witte spikkels op voorfemur afwezig; vleugelfranje bij inmonding van 3de long. licht ....14.

14 a Vleugelfranje bij inmonding van den boventak der 4de long. donker; witte schubben ventraal op 2de-7de abdominaalsegment aanwezig .... **A. barbirostris.**

b Vleugelfranje bij inmonding van den boventak der 4de long. licht; witte schubben ventraal op 2de—7de abdominaalsegment afwezig .... **A. pseudumbrosus.**

15 a Palpen met drie lichte ringen, maar top donker; lichte franjetop alleen bij inmonding van boventak der 2de long. **A. gigas.**

b Palpen met drie of vier lichte ringen, top licht; franjetop licht, tot minstens bij inmonding der 3de long. ....16.

16 a Tarsale 4 en 5 van den achterpoot wit (soms wat bruin aan top 5 of midden op 4) .... **A. sinensis var. argyropus.**

b Geen der tarsalia geheel wit ....17.

17 a Geen ventrale schubbenbundel op 7de abdominaalsegment; goudgele franjetop van even voor inmonding van ondertak der 2de long. tot en met 3de long. .... **A. separatus.**

b Ventrale schubbenbundel op 7de abdominaalsegment; goudgele franjetop van vòòr inmonding der 1ste tot voorbij die der 3de long. .... **A. sinensis.**

18 a Tarsale 5 van den achterpoot geheel wit ....19.

b Geen der tarsalia geheel wit ....23.

19 a Tarsalia 3 t/m 5 van den achterpoot geheel wit ....20.

b Tarsale 3 niet geheel wit ....21.

20 a Pooten gevlekt .... **A. ramsayi.**

b Pooten ongevlekt; tarsale 2 van den achterpoot apicaal over 1/5 — 1/2 der lengte wit .... **A. philippinensis.**

21 a Tarsalia 4 en 5 van den achterpoot geheel wit **A. schüffneri.**

b Alleen tarsale 5 van den achterpoot geheel wit ....22.

22 a Pooten gevlekt .... **A. maculatus.**

b Pooten ongevlekt .... **A. karwari.**

23 a Pooten niet duidelijk geband (loupe-vergrooting!); palpen met niet meer dan drie lichte ringen .... 24.

b Pooten duidelijk geband (soms slechts gedeeltelijk) ......25.

24 a Proboscis voor distale helft licht .... **A. aconitus.**

b Proboscis geheel donker .... **A. minimus.**

25 a Palpen met meer dan drie lichte ringen; pooten gevlekt 26.

b Palpen met drie lichte ringen; pooten al dan niet gevlekt 31.

26 a Ventraal apicaal op 2de—7de abdominaalsegment zes paar *zwarte* schubbenbundels; op het 8ste abdominaalsegment ventraal gele schubben. .... **A. kochi.**

b Geen ventrale abdominale schubbenbundels ....27.

27 a Breede schubben over het *geheele* mesonotum, met gele haren daartusschen .... 28.

b Haarvormige òf vrij breede kromme schubben *alleen op den voorrand van het mesonotum*, overigens haren ....29.

28 a Proboscis gedeeltelijk licht .... **A. punctulatus.**

b Proboscis meestal geheel donker met lichte vlek onder oliva .... **A. punctulatus var. moluccensis.**

29 a Proboscis veel langer dan palpen (17:14); de basis[1]) der 2de vorkcel ligt dichter bij de vleugelbasis dan de basis der 1ste vorkcel; achterpoot sterk gevlekt (t/m 3de tarsale) ......30.

b Proboscis vrijwel even lang als de palpen; de basis der 2de vorkcel ligt verder van de vleugelbasis dan de basis der 1ste vorkcel; achterpoot minder sterk gevlekt (niet verder dan 1ste tarsale) .... **A. tessellatus.**

30 a De apicale gele banden aan de bruingele tarsalia 1 t/m 4. moeilijk te zien (vooral aan den middenpoot) **A. longirostris.**

b De apicale gele banden aan de zwarte tarsalia 1 t/m 4 door het kleurverschil veel duidelijker .... **A. longirostris var. annulatus.**

31 a Pooten meer of minder sterk gevlekt ....32.

b Pooten ongevlekt ....34.

32 a Op costa bleeke plekken grooter dan de zwarte vlekken;zeer sterk gevlekte pooten (ook de tarsalia 2 t/m 4) **A. parangensis.**

b Op costa bleeke plekken kleiner dan de zwarte vlekken;minder sterk gevlekte pooten (tarsalia 3 en 4 niet gevlekt) ...33.

33 a Vleugel helderder (komt in zeer veel opzichten met den vleugel van A. rossii overeen); de 2de distale costavlek is ongeveer even lang als de voorafgaande lichte plek en iets kleiner dan de opvolgende lichte plek; de boventak der 5de long. heeft drie kleine vlekken [verhouding van donker tot geheele lengte der boventak als 30 (max. 40):100]; palpen bruin, de lichte top is zeer breed, 3—5 x zoo lang als de daaropvolgende donkere band .... **A. ludlowi.**

b Vleugel donkerder; de 2de distale costavlek is 1,5 tot 3 x zoo lang als de voorafgaande plek en staat in dezelfde verhouding tot de volgende lichte plek; boventak der 5de long. heeft drie (soms twee, door versmelting der twee distale) groote vlekken [verhouding van donker tot geheele lengte der boventak als ruim 40, meestal 50 (max. 88!):100]; palpen donkerder bruin, de lichte top meestal even lang, hoogstens

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[1]) Onder „basis" van een vorkcel verstaat men dat gedeelte, hetwelk het dichtst bij de vleugelbasis gelegen is, dus de bifurcatie-plaats der betreffende longitudinale.

2 × zoo lang, als de daarop volgende donkere band .... **A.ludlowi var. sundaica.**

34 a Proboscis op oliva na geheel donker; lichte palpentop nagenoeg even breed als daaronder gelegen zwarte ring (variaties: 1—3 × zoo breed) .... **A.rossii.**

b Proboscis onder oliva nagenoeg steeds met lichte plek; lichte palpentop 3—5 × zoo breed als daaronder gelegen zwarte ring (variaties van 1—11 × zoo breed) .... **A.vagus.**

Nieuwe benamingen:

A.bironelli: vroeger: Bironella gracilis.

A.philippinensis: tot deze eene soort zijn vereenigd de beide subspecies van Nyssorhynchus fuliginosus, nl. N. f. type en N. f. var. nivipes.

A.pseudumbrosus: vroeger: Myzorhynchus barbirostris var. pallidus Swellengrebel.

A.ramsayi: vroeger: abusievelijk als Nyssorhynchus jamesi beschreven.

A.separatus: vroeger: Myzorhynchus sinensis var. separatus. Voorts wordt in de buitenlandsche nomenclatuur *A.sinensis* tegenwoordig vaak als *A.hyrcanus* en *A.rossii* als *A.subpictus* aangeduid.

Alphabetische volgorde der vrouwelijke Anophelinen (38 soorten; het achter den naam geplaatste cijfer geeft het nummer der tabel aan, in hetwelk deze soort behandeld wordt).

1	A.aconitus	24a.
2	A.aitkenii	6a.
3	A.albotaeniatus	11a.
4	A.amictus	1a.
5	A.annandalei [1]	7a.
6	A.bancrofti	13a.
7	A.barbirostris	14a.
8	A.bironelli	4a.
9	A.brevipalpis	6b.
10	A.errabundus	1b.

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[1] Zie noot 1 op p. 378.

11	A.gigas	15a.
	(A.hyrcanus: zie A. sinensis)	
12	A.immaculatus	5a.
13	A.karwari	22b.
14	A.kochi	26a.
15	A.leucosphyrus type	8a.
16	A.leucosphyrus var. hackeri	8b.
17	A.longirostris	30a.
18	A.longirostris var. annulatus	30b.
19	A.ludlowi	33a.
20	A.ludlowi var. sundaica	33b.
21	A.maculatus	22a.
22	A.minimus	24b.
23	A.papuae	4c.
24	A.parangensis	32a.
	A.peditaeniatus: als imago nog niet in Ned.-Indië gevonden.	
25	A.philippinensis	20b.
26	A.pseudumbrosus	14b.
27	A.punctulatus	28a.
28	A.punctulatus var. moluccensis	28b.
29	A.ramsayi	20a.
30	A.rossii (=A.subpictus : buitenlandsche nomenclatuur)	34a.
31	A.schüffneri	21a.
32	A.separatus	17a.
33	A.sinensis (= hyrcanus:buitenlandsche nomenclatuur)	17b.
34	A.sinensis var. argyropus	16a.
35	A.tessellatus	29b.
36	A.travestitus	4b.
37	A.umbrosus	12a.
38	A.vagus	34b.

II. Tabel voor determinatie der mannetjes-Anophelinen.

1a Abdomen dorsaal van 2de t/m 8ste segment dicht bezet met breede goudgele schubben, met een groep van donkere schubben midden op de segmenten 4—6; ventraal op 2de t/m 8ste segment iets minder dicht bezet (naar de bases der segmenten toe) met breede witte èn gele schubben; proboscis geheel donker; de vleugelcosta met zes groote donkere vlekken, de 1ste long. met 14—18 kleine zwarte vlekken, geen groote vlekken (beschrijving volgens Edwards) .... **A. amictus.**

b Abdomen niet aldus .... 2.

2a Vleugel ongevlekt .... 3.

b Vleugel gevlekt .... 7.

3a De stam der 4de en de boventak der 5de long. zijn gegolfd 4.

b Niet aldus .... 5.

4a Palpen langer dan de halve lengte van de proboscis; proboscis belangrijk korter dan de voorfemur; de eerste vorkcel meet ongeveer 1/5 van de lengte van den stam der 2de long.; antennen pluimvormig .... **A. bironelli.**

b Palpen meten ongeveer 1/4 van de lengte van de proboscis; proboscis langer dan de voorfemur; de eerste vorkcel meet ongeveer 4/5 van de lengte van den stam der 2de long.; de antennen geheel gelijk aan die van het wijfje (♂ en ♀ alleen aan de genitaliën te onderscheiden) .... **A. travestitus.**

c Palpen nagenoeg even lang als de proboscis; proboscis iets korter dan de voorfemur (verhouding als 63:71); de eerste vorkcel bestaat feitelijk niet, daar de ondertak der 2de long. niet in den boventak uitmondt; de stam der 4de long. heeft op de benedenwaartsche uitbochting, welke gelegen is halverwege top 2de vorkcel en achterste dwarsader, op den top dier bocht een verdikking dier ader .... **A. papuae.**

5a Gele vleugel (proboscis roomwit, palpen roomwit tot licht geel) .... **A. immaculatus.**

b Bruine vleugel ....6.

6a Kop met zeer smalle vorkschubben; prothoraxlobben zonder schubbenbundel; palpen dun, grijsbruin, ongeband, even lang als proboscis .... **A. aitkenii.**

b Kop met breede vorkschubben; prothoraxlobben vooraan met schubbenbundel; palpen bijna even lang als proboscis .... **A. brevipalpis.**

7a Achterpoot vertoont op het tibio-femoraal-gewricht een, reeds met het bloote oog zichtbare, breede krans van zilverwitte uitstaande schubben, waarboven een nog iets breedere krans van gitzwarte meer tegen den femur aanliggende schubben; costa geheel bruin, zonder lichte plekken, franjetop geelwit van even vòòr inmonding der eerste long. tot even voorbij inmonding van boventak der tweede long .... **A. annandalei.**

b Achterpoot met breeden lichten tibiotarsalen band .... **A. leucosphyrus** en **A. leucosphyrus var. hackeri.**

c Achterpoot anders dan onder a. en b. beschreven ....8.

8 a Costa (gerekend tot inmonding der eerste long.) met hoogstens twee duidelijke lichte plekken ....9.

b Costa met meer dan twee duidelijke lichte plekken ...... 16.

9 a Tarsale 5 achterpoot wit (hoogstens top even bruin) ... 10.

b Geen der tarsalia wit .... 11.

10 a Alleen tarsale 5 wit .... **A. albotaeniatus.**

b Tarsale 4 èn 5 wit (soms even bruin aan den top van 5 en in het midden van 4) .... **A. sinensis var. argyropus.**

11 a Palpen zwart òf bruin en zwart .... 12.

b Palpen met wit en zwart .... 15.

12 a Ventraal op 2de—7de abdominaalsegment mediaan (en soms ook lateraal) een paar witte schubbenbundels, èn (òf) een groep van witte schubben (3—20) èn (òf) witte haren (3—4) op den achterbeugel .... 13.

b Geen witte abdominale schubbenbundels; geen groep van witte schubben en witte haren op den achterbeugel .... **A. umbrosus.**

13 a Witte spikkels op voorfemur (muskiet van onderen bekijken); vleugelfranje bij inmonding der derde long. donker; de witte schubben op den achterbeugel vaak geheel afwezig, dikwijls zijn er slechts 1, 2 of 3 .... **A. bancrofti.**

b Witte spikkels op voorfemur afwezig; vleugelfranje bij inmonding der derde long. licht .... 14.

14 a Witte ventrale schubbenbundels op 2de—7de abdominaalsegment aanwezig; vleugelfranje bij inmonding van boventak der vierde long. donker .... **A. barbirostris.**

b Geen witte ventrale schubbenbundels op 2de—7de abdominaalsegment; vleugelfranje bij inmonding van boventak der vierde long. licht .... **A. pseudumbrosus.**

15 a Vleugelfranje aan top licht allèèn bij inmonding van boventak der tweede long. .... **A. gigas.**

b Vleugelfranje aan top licht van even voor inmonding van ondertak der tweede tot en met die der derde long. **A. separatus.**

c Vleugelfranje aan top licht van vòòr inmonding der eerste tot voorbij die der derde long. .... **A. sinensis.**

16 a Tarsale 5 van den achterpoot geheel wit .... 17.

b Geen der tarsalia geheel wit .... 20.

17 a Tarsale 3—5 van den achterpoot geheel wit .... 18.

b Tarsale 4 en 5 van den achterpoot geheel wit **A. schüffneri.**

c Alleen tarsale 5 van den achterpoot geheel wit .... 19.

18 a Pooten gevlekt .... **A. ramsayi.**

b Pooten ongevlekt; tarsale 2 van den achterpoot apicaal over 1/5—1/2 der lengte wit .... **A. philippinensis.**

19 a Pooten gevlekt .... **A. maculatus.**

b Pooten ongevlekt .... **A. karwari.**

20 a Pooten niet duidelijk geband ...... **A. aconitus** òf **A. minimus.**

b Pooten duidelijk geband (soms slechts gedeeltelijk) ...... 21.

21 a Apicaal ventraal op 2de—7de abdominaalsegment 6 paar *zwarte* schubbenbundels; op het 8ste segment gele schubben .... **A. kochi.**

b Geen abdominale schubbenbundels .... 22.

22 a Over het geheele mesonotum breede witgele schubben, waartusschen gele haren .... **A. punctulatus,** òf **A. punctulatus var. moluccensis.**

b Geen breede schubben over het *geheele* mesonotum, *alleen op den voorrand schubben* (haarvormige òf vrij breede kromme schubben) .... 23.

23 a 3de long. met veel (4—6) donkere vlekken .... 24.

b 3de long. bijna geheel licht (van dwarsader af tot vleugelrand hoogstens twee kleine donkere vlekjes)............... 26.

24 a Achterpoot sterk gevlekt (tot en met 3de tarsale) ...... 25.

b Achterpoot minder sterk gevlekt (niet verder dan 1ste tarsale) .... **A. tessellatus.**

25 a De apicale gele banden aan de bruingele tarsalia 1 t/m 4 moeilijk te zien (vooral aan den middenpoot) .... **A. longirostris.**

b De apicale gele banden aan de zwarte tarsalia 1 t/m 4 door het kleurverschil veel duidelijker .... **A. longirostris var. annulatus.**

26 a Pooten ongevlekt; op boventak der 5de long. 3 kleine vlekken .... 27.

b Pooten gevlekt .... 28.

27 a Op het gewricht tusschen 4de en 5de tarsale der voorpooten een vrij breede witte band, overgrijpende op beide tarsalia, dus op voortarsalia *drie duidelijke* banden .... **A. rossii.**

b Op het gewricht tusschen 4de en 5de tarsale der voorpooten een *zeer* smalle witte band, bijna steeds beperkt tot één (meestal het 5de) tarsale, dus op voortarsalia *twee duidelijke* banden .... **A. vagus.**

28a Op costa bleeke plekken grooter dan de zwarte vlekken; zeer sterk gevlekte pooten (ook de tarsalia 2 t/m 4)... **A.parangensis.**

b Op costa bleeke plekken kleiner dan de zwarte vlekken; minder sterk gevlekte pooten (tarsalia 3 en 4 niet gevlekt), ... **A.ludlowi** òf **A. ludlowi var. sundaica.**

Nieuwe benamingen:

A.bironelli: vroeger: Bironella gracilis.

A.philippinensis: tot deze eene soort zijn vereenigd de beide subspecies van Nyssorhynchus fuliginosus, nl. N.f. type en N.f. var. nivipes.

A.pseudumbrosus: vroeger: Myzorhynchus barbirostris var. pallidus Swellengrebel.

A.ramsayi: vroeger abusievelijk als Nyssorhynchus jamesi beschreven.

A.separatus: vroeger: Myzorhynchus sinensis var. separatus. Voorts wordt in de buitenlandsche nomenclatuur *A.sinensis* tegenwoordig vaak als *A.hyrcanus* en *A.rossii* als *A.subpictus* aangeduid.

Alphabetische volgorde der mannelijke Anophelinen (35 soorten; het achter den naam geplaatste cijfer geeft het nummer der tabel aan, in hetwelk deze soort behandeld wordt).

1	A.aconitus	20a.
2	A.aitkenii	6a.
3	A.albotaeniatus	10a.
4	A.amictus	1a.
5	A.annandalei [1]	7a.
6	A.bancrofti	13a.
7	A.barbirostris	14a.
8	A.bironelli	4a.
9	A.brevipalpis	6a.
	(A.errabundus: mannetje nog onbekend).	
10	A.gigas	15a.
	(A.hyrcanus: zie A.sinensis).	

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[1] Zie noot 1 op p. 378.

11	A.immaculatus	5a.
12	A.karwari	19a.
13	A.kochi	21a.
14	A.leucosphyrus èn A.leucosphyrus var. hackeri	7b.
15	A.longirostris	25a.
16	A.longirostris var. annulatus	25b.
17	A.ludlowi èn A.ludlowi var. sundaica	28b.
18	A.maculatus	19a.
19	A.minimus	20a.
20	A.papuae	4c.
21	A.parangensis	28a.
	A.peditaeniatus: als imago nog niet in Ned.-Indië gevonden.	
22	A.philippinensis	18b.
23	A.pseudumbrosus	14b.
24	A.punctulatus	22a.
25	A.punctulatus var. moluccensis	22a.
26	A.ramsayi	18a.
27	A.rossii (=A.subpictus: buitenlandsche nomenclatuur)	27a.
28	A.schüffneri	17b.
29	A.separatus	15b.
30	A.sinensis (= A.hyrcanus: buitenlandsche nomenclatuur)	15c.
31	A.sinensis var. argyropus	10b.
32	A.tessellatus	24b.
33	A.travestitus	4b.
34	A.umbrosus	12b.
35	A.vagus	27b.

III. Tabel voor determinatie der Anophelinen-larven.

1 a Antennen proximaal met mediaal geplaatst vertakt haar; middenclypeusharen bijna steeds vlak bij elkaar, soms even ver van elkaar als van de zijclypeusharen (zie 9b en 9c). 2.

b Antennen proximaal zonder vertakt haar; middenclypeusharen niet vlak bij elkaar .... 12.

2 a Op thorax twee paar onvolkomen waaiers [1]) .... 3.

b Op thorax een paar waaiers of geen waaiers .... 4.

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[1]) Het voorste paar op de grens van voorste en volgende —, het achterste paar op de grens van achterste en voorgaande thorax-quadrant: zoeken bij 200-voudige vergr. (bij levende larven duidelijker dan bij de gemonteerde exemplaren !).

3 a Zijclypeusharen 2/3—4/5 van de lengte der middenclypeusharen, meer of min vertakt (2—8 takjes); binnenschouderhaar zonder duidelijken wortel met 3—8 takjes **A. bironelli.**

b Zijclypeusharen 0,5—0,7 van de lengte der middenclypeusharen, onvertakt, hoogstens op halve hoogte gevorkt; binnenschouderhaar met 11—16 takjes .... **A. travestitus.**

c Zijclypeusharen zeer kort, 0,08—0,14 van de lengte der middenclypeusharen, twee- tot vijfmaal gevorkt, soms onvertakt ; binnenschouderhaar zeer kort, plomp, aan den top in twee tot vijf takken gesplitst .... **A. papuae.**

4 a Geen waaiers op den thorax .... 5.

b Een paar waaiers [1]) op den thorax .... 7.

5 a Geen waaiers op de abdominaalsegmenten .:................ 6.

b Alleen, en wel volkomen, waaiers op de abdominaalsegmenten 3 t/m 7 (dus op segment 1 en 2 geen waaiers) **A.gigas.**

c Alleen, en wel volkomen, waaiers [2]) op het 4de en 5de abdominaalsegment (dus op de overige segmenten geen waaiers) .... **A. novumbrosus.**

6 a Antennehaar met 7—13 zijtakken; zijclypeushaar met 5—8 (tot 13) zijtakken; middenschouderhaar met 4—9 zijtakken .... **A.umbrosus.**

b Antennehaar met 17—26 zijtakken; zijclypeushaar met 11—16 zijtakken; middenschouderhaar met 7—12 zijtakken ...... **A.separatus.**

7 a Aantal takken der zijclypeusharen 40 of meer .... 8.

b Aantal takken der zijclypeusharen 25 of minder .... 9.

8 a Binnenschouderhaar onvertakt, òf met 1—4 aan den top ontspringende takjes **A. sinensis**, en **idem, var. argyropus.**

b Binnenschouderhaar met 3—10 takjes, vlak aan de basis ontspringend; middenclypeusharen onbehaard en onvertakt; achterclypeusharen kort, met 2—5 zijtakjes ... **A.barbirostris.**

c Binnenschouderhaar met 3—5 takken, die eerst op ± 1/3 der hoogte beginnen te ontspringen; middenclypeusharen met ± 8 zijhaartjes (die soms iets grof en lang zijn) op de bovenste helft; achterclypeusharen bijna nooit vertakt, zeer zelden gevorkt .... **A. bancrofti.**

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[1]) Slechts *kleine onvolkomen* waaiers (behalve bij *aitkenii var. palmata*: zie 11b) met 6—22 smalle lange nagenoeg steeds ongezaagde bladen, gelegen op de grens van achterste en voorgaande thorax-quadrant: zoeken bij 200-voudige vergr. (bij levende duidelijker dan bij de gemonteerde exemplaren !).

[2]) Reeds gemakkelijk te zien bij 50-voudige vergr. (ook bij de gemonteerde larven !).

9 a Middenclypeusharen vlak bij elkaar, onvertakt en onbehaard .... 10.

b Middenclypeusharen even ver van elkaar als van de zijclypeusharen, onvertakt en onbehaard; zijclypeusharen met 12—24 takken .... **A. albotaeniatus.**

c Middenclypeusharen òf even ver van elkaar als van de zijclypeusharen, op 1/3 der hoogte zich in 2—7 takken splitsend en meestal onbehaard, òf onvertakt, dan wel op ± halve hoogte gevorkt en meestal behaard, en dichter bij elkaar dan bij de zijclypeusharen; zijclypeusharen onvertakt, òf op zeer wisselende hoogte in 2—5 takken gesplitst... **A. aitkenii type.**

10 a Zijclypeusharen dicht bij de basis zich splitsend in 2—3 takken, die zich verderop weer deelen, in totaal 8—17 takjes .... **A. pseudumbrosus.**

b Zijclypeusharen op 1/3—1/2 der hoogte bezemvormig vertakt (6—9 takken); middenschouderhaar met 8 takken .... **A. peditaeniatus**[1].

c Zijclypeusharen onvertakt, òf in 2 takjes gesplitst; middenschouderhaar met 12—16 takken .... 11.

11 a Zijclypeusharen onvertakt; binnenschouderhaar met 6—8 takjes; op den thorax een paar onvolkomen waaiers met ± 12 (6—17) smalle lange bladen... **A. aitkenii var. insulae florum.**

b Zijclypeusharen onvertakt, òf dicht boven den wortel in twee takjes gesplitst; binnenschouderhaar is een soort waaier, met 18—19 fijne haarachtige bladen; op den thorax een paar volkomen waaiers[2] met 18—19 breede, scherp gezaagde bladen met lange scherpe punt; groote bijschilden op alle segmenten .... **A. aitkenii var. palmata.**

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[1] Hiervan is tot nu toe *alléén de larve* in Ned.-Indië gevonden, en slechts als één enkel exemplaar, einde 1929 op het eiland BANKA, in de omgeving van Muntok (Verzameling Malaria-Afdeeling, Geneeskundig Laboratorium, Salemba, Weltevreden).

[2] Even krachtig ontwikkeld als die op de abdominaalsegmenten (in tegenstelling met de andere Anophelinen-larven, die thoraxwaaiers bezitten, welke dan onvolkomen en zwakker ontwikkeld zijn).

12 a Antennen met een mediaal klein onvertakt haar, geplaatst op ± 3/5 van de hoogte der antenne .... **A. brevipalpis.**

b Antennen zonder eenig mediaal antennenhaar (wel met een zeer klein onvertakt lateraal haar) .... 13.

13 a Binnenschouderhaar met duidelijken wortel en dikke schacht .... 14.

b Binnenschouderhaar zonder duidelijken wortel of met matigen wortel, en dunne schacht (soms bij ludlowi ook vrij duidelijke wortel: bij ludlowi echter geen waaiers op den thorax!). .... 25.

14 a Zijclypeusharen meer of minder sterk bezemvormig vertakt .... 15.

b Zijclypeusharen onvertakt, òf met enkele (2 tot 3) zijtakken, òf onvertakt maar behaard .... 16.

15 a Middenclypeusharen lang, onvertakt en onbehaard, zelden aan den top even gespleten in 2 of 3 takjes; zijclypeusharen op halve hoogte in 5—10 takjes splitsend; achterclypeusharen zeer kort, onvertakt (soms aan den top in tweeën of drieën gespleten), met geringe achterwaartsche plaatsing ongeveer halverwege tusschen midden- en zijclypeusharen insereerende; frontaalharen geheel afwijkend van alle overige Anophelinen-larven, nl.: binnenhaar lang, onvertakt, onbehaard; middenhaar ± 1/4—1/3 van het binnenhaar, onvertakt òf zich op 2/3 der hoogte in 3 takjes splitsend; buitenhaar langer dan middenhaar, op 1/3 der hoogte zich in 4—6 takken splitsend; thorax en alle abdominaalsegmenten, afwijkend van alle andere Anophelinen-larven, dicht bezet met korte, krachtige doornachtige uitsteeksels, reeds bij 50-voudige vergrooting zeer opvallend ...... **A. annandalei** [1].

b Middenclypeusharen lang, onvertakt, met vrij lange en krachtige haartjes (9—13); zijclypeusharen dicht boven de basis sterk bezemvormig vertakt (10—22 takjes); achterclypeusharen 1/3 van de middenclypeusharen, met 3—6 basaal ontspringende takjes, groote achterwaartsche plaatsing, iets verder uit elkaar dan de middenclypeusharen .... **A. philippinensis.**

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[1] Het is gebleken, dat de door S. L. Brug (1926) beschreven *A. annandalei var. djajasanensis* geheel identiek is met den door Baini Prashad (1918) beschreven *A. annandalei*, zoodat slechts deze naam mag blijven bestaan.

c Middenclypeusharen lang, onvertakt, met langere en talrijker haartjes (± 30); zijclypeusharen als bij A.philippinensis; achterclypeusharen 1/4 van de middenclypeusharen, onvertakt, òf aan den top gevorkt, iets naar binnen van- en vrij ver achter de zijclypeusharen insereerende... **A. schüffneri.**

16 a Achterclypeusharen kort en vertakt, òf lang en onvertakt, in welk geval de midden- en zijclypeusharen onbehaard ... 17.

b Achterclypeusharen meestal onvertakt 1), maar dan toch bijna altijd middenclypeusharen behaard (hoewel soms slechts fijn en spaarzaam) .... 19.

17 a Op de abdominaalsegmenten dorsaal en proximaal groote gele doorschijnende bijschilden, die heel zelden nagenoeg onzichtbaar kunnen zijn; binnenschouderhaar met 16—26-, middenschouderhaar met 11—16 zijtakken; op thorax een paar onvolkomen waaiers met ± 12 bladen .... 18.

b Geen groote doorschijnende bijschilden dorsaal en proximaal op de abdominaalsegmenten; binnenschouderhaar met ± 15 -, middenschouderhaar met 18—22 takken; middenclypeusharen lang, ver uit elkaar, meestal onvertakt en onbehaard; zijclypeusharen normaal onvertakt, echter ook zeer vaak gevorkt (direct boven den wortel of slechts aan den top), òf met 2 of 3 zijtakken; op thorax beiderzijds geen waaier, maar een veer met 4—5 takken **A. parangensis.**

18 a Midden- en zijclypeusharen onvertakt en fijn behaard (soms zijclypeusharen onvertakt èn onbehaard); achterclypeusharen kort, nabij de basis in 2—5 takken gesplitst **A. aconitus type 1.**

b Midden- en zijclypeusharen onvertakt en onbehaard; achterclypeusharen lang, onvertakt en onbehaard. ....
A. aconitus type 2 — A. minimus.

19 a Een paar waaiers op thorax .... 20.

b Geen waaiers op thorax; midden- en zijclypeusharen behaard .... 24.

20 a Zijclypeusharen minstens 1/2 van de lengte der middenclypeusharen, onvertakt, maar met duidelijke zijharen ... 21

b Zijclypeusharen korter, onvertakt en nagenoeg steeds onbehaard (bij zeer hooge uitzondering van een paar fijne haren voorzien).. 22.

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1) Alleen bij *A.longirostris var. annulatus* steeds sterk vertakt (zie 21b), en bij *A.punctulatus var. moluccensis* en *A.karwari* hoogst zelden gevorkt.

21 a Achterclypeusharen kort en onvertakt, hoogst zelden gevorkt; de wortels van het binnen- en middenschouderhaar zijn zwaar en steeds met elkaar vergroeid .... **A.punctulatus var. moluccensis.**

b Achterclypeusharen lang en sterk vertakt (7—9 takken); de wortels van het binnen- en middenschouderhaar zijn zwaar, maar nooit met elkaar vergroeid .... **A.longirostris var. annulatus.**

22 a De zware wortels van binnen- en middenschouderhaar zijn vaak met elkaar vergroeid; op 2de abdominaalsegment een paar rudimentaire waaiers met 6—16 haren en smalle bladen... **A.leucosphyrus type**, en **A.leucosphyrus var. hackeri.**

b De wortels van binnen- en middenschouderhaar zijn nooit met elkaar vergroeid; op 2de abdominaalsegment een paar gewone waaiers (met 11—16-, resp. 18—22 bladen) .... 23.

23 a Binnenoccipitaalharen steeds onvertakt; het binnenschouderhaar heeft 9—14 takken, en, evenals het middenschouderhaar, een duidelijken niet gepigmenteerden wortel; op het 2de abdominaalsegment een paar volkomen waaiers met 18—22 weinig gepigmenteerde bladen, gezaagd, maar zonder draad .... **A.punctulatus.**

b Binnenoccipitaalharen met 2—4 takken; het binnenschouderhaar heeft 14—20 takken, en, evenals het middenschouderhaar, een zwaren, sterk gepigmenteerden wortel; op het 2de abdominaalsegment een paar gewone waaiers met 11—16 bladen, niet gezaagd, maar met een draad...... **A.longirostris.**

24 a Waaierbladen op middelste abdominaalsegmenten met spitse punten .... **A.maculatus.**

b Waaierbladen op middelste abdominaalsegmenten met stomper en korter punten .... **A.karwari.**

25 a Korte achterclypeusharen, vlak achter en binnenwaarts van de middenclypeusharen, nog voorbij den voorsten clypeusrand uitstekend .... **A.vagus- A.rossii type 1.**

b Achterclypeusharen ver achter de middenclypeusharen, even ver of verder van elkaar dan deze .... 26.

26 a Waaiers of waaierharen op thorax .... 27.

b Geen waaiers op thorax .... 28.

27 a Binnenschouderhaar zeer donker, kort (veel kleiner dan de halve lengte van het middenschouderhaar), met weinig (2—6) takken; binnenoccipitaalharen meestal met 2—4

takken (soms onvertakt); zijclypeusharen onvertakt en onbehaard, ± 1/5 van de middenclypeusharen (bij de levende larve moeilijk zichtbaar!) .... **A.tessellatus.**

b Binnenschouderhaar licht, kort (± 1/2 van het middenschouderhaar) met 8—10 takken; binnenoccipitaalharen onvertakt; zijclypeusharen met 4—10 korte, stekelachtige takjes, 0,6—0,7 van de middenclypeusharen ...... **A.ramsayi.**

c Binnenschouderhaar licht, lang (0,53—0,63 van het middenschouderhaar), met 5—11 takken; binnenoccipitaalharen meestal onvertakt; korte, onvertakte en onbehaarde zijclypeusharen, 0,2—0,38 van de middenclypeusharen... **A.kochi.**

28 a Middenclypeusharen met enkele zijhaartjes, zijclypeusharen met 4 lange zijharen; binnenschouderhaar met 2—4, middenschouderhaar met 7—9 takken .... **A.amictus.**

b Clypeusharen onbehaard; binnenschouderhaar met ± 11, middenschouderhaar met ± 15 takken ....
A.ludlowi- A.rossii type 2.

Nieuwe benamingen:

A.bironelli: vroeger: Bironella gracilis.

A.philippinensis: vroeger: Nyssorhynchus fuliginosus.

A.pseudumbrosus: vroeger: Myzorhynchus barbirostris var. pallidus Swellengrebel.

A.ramsayi: vroeger abusievelijk als Nyssorhynchus jamesi beschreven.

A.separatus: vroeger: Myzorhynchus sinensis var. separatus. Voorts wordt in de buitenlandsche nomenclatuur *A.sinensis* tegenwoordig vaak als *A.hyrcanus* en *A.rossii* als *A.subpictus* aangeduid.

Alphabetische volgorde der Anophelinen-larven (36 soorten: het achter den naam geplaatste cijfer geeft het nummer der tabel aan, in hetwelk deze soort behandeld wordt).

1 A.aconitus type 1 .... 18a.
2 A.aconitus type 2 - A.minimus .... 18b.
3 A.aitkenii type .... 9c.
4 A.aitkenii var. insulae florum .... 11a.
5 A.aitkenii var. palmata .... 11b.
6 A.albotaeniatus .... 9b.

7	A.amictus	28a.
8	A.annandalei[1]	15a.
9	A.bancrofti	8c.
10	A.barbirostris	8b.
11	A.bironelli	3a.
12	A.brevipalpis	12a.
	A.errabundus: larve nog onbekend.	
13	A.gigas	5b.
	A.hyrcanus: zie A.sinensis.	
14	A.karwari	24b.
15	A.kochi	27c.
16	A.leucosphyrus en A.leucosphyrus var. hackeri	22a.
17	A.longirostris	23b.
18	A.longirostris var. annulatus	21b.
19	A.ludlowi - A.rossii type 2	28b.
20	A.maculatus	24a.
	A.minimus: zie A.aconitus type 2.	
21	A.novumbrosus	5c.
22	A.papuae	3c.
23	A.parangensis	17b.
24	A.peditaeniatus	10b.
25	A.philippinensis	15b.
26	A.pseudumbrosus	10a.
27	A.punctulatus	23a.
28	A.punctulatus var. moluccensis	21a.
29	A.ramsayi	27b.
	A.rossii type 1: zie A.vagus.	
	A.rossii type 2: zie A.ludlowi.	
30	A.schüffneri	15c.
31	A.separatus	6b.
32	A.sinensis (= A.hyrcanus: buitenlandsche nomenclatuur)	8a.
	A.sinensis var. argyropus: zie A.sinensis.	
	A.subpictus: zie A.rossii.	
33	A.tessellatus	27a.
34	A.travestitus	3b.
35	A.umbrosus	6a.
36	A.vagus - A.rossii type 1	25a.

Weltevreden, 15 Januari 1930.

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[1] *Zie noot 1, p. 378.*

Anne
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EFFECTS OF DIETARY MODIFICATIONS OF HOST RATS ON THE TAPEWORM HYMENOLEPIS DIMINUTA[1]

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I. USE OF TRIBOLIUM CONFUSUM AS A VECTOR; LIMITATION OF THE AMOUNT OF FOOD

Among the results on the effects of intestinal parasites of starvation of the host is that reported by Levine (35), who observed that the number of discharged segments of the poultry cestode *Davainea proglottina* was decreased for a period of a week following 24 hours of starvation of the hosts. On the other hand, Hegner and Eskbridge (26) kept six rats showing infections with intestinal trichomonads without food for eight days, and, at the end of this period, all the hosts were still infected and four of the infections were considered heavy.

In the investigation here reported, fifteen young rats of the Wistar A inbred strain[2] were placed in separate cages of hardware cloth and supplied freely with water and a ration made up as follows: beet sugar, 63 per cent; casein[3], 15 per cent; Celluflour, 3 per cent; complete salt mixture[4], 4 per cent; cod liver oil, 2 per cent; lard, 3 per cent; Fleischmann's yeast, 10 per cent.

After a preliminary examination of the pellets in which no tapeworm eggs were found, the animals were fed cysticercoids of *Hymenolepis diminuta* through the use of the flour beetle *Tribolium confusum* as a vector. Gravid proglottids from the posterior portion of rat tapeworms were mashed with a small amount of wheat middlings and furnished as food to confused flour beetles. After a period of three weeks to permit the development of cysticercoids in the intermediate hosts, the heads were removed from the beetles thus exposed to infection and both bodies and heads of about a dozen beetles added to the food of each rat. In order to insure prompt ingestion of the vectors, rats were deprived of food for a half day, then given only a small amount of food containing the dead beetles.

Fifteen days after the feeding of the intermediate hosts, enameled pans were placed under the cages. The one-half inch mesh of the hardware cloth used for the floors of the cages permitted the rat pellets to drop through and be caught in the pans containing half an inch of water in which was dissolved a small amount of trisodium phosphate which softened the pellets somewhat and retarded bacterial growth. Every two days the pans were removed and replaced by clean ones, and the contents of each pan, containing the entire number of pellets for the preceding two days, were examined for tapeworm eggs. Three of the fifteen rats did not show eggs in the droppings by the thirtieth day and were considered to be uninfected and were not used further. The other twelve began to eliminate eggs within 17 to 24 days after ingestion of the cyticercoids.

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[1] This series of papers is a part of Doctoral thesis No. 513 deposited in the Iowa State College Library, Ames, Iowa.

[2] Obtained from the Foods and Nutrition Laboratory of the Home Economics Division of the Iowa State College.

[3] Vitamin-free casein obtained from Casein Company of America, New York.

[4] Complete inorganic salt mixture obtained from Harris Laboratory, Tuckahoe, New York.

The collected pellets were broken up and the material from each pan was thoroughly mixed with an electric beater and diluted with water to one liter. This suspension was then poured from one container to another to insure, insofar as possible, a homogeneous mixture. Immediately a small sample was poured off and thoroughly mixed; from it a small amount of fluid was drawn into a pipette fitted with a rubber bulb, and dropped into a mold counting chamber of known volume. The entire area of the counting chamber was examined and the number of eggs present determined, from which the number of eggs in the entire liter of material, and the number eliminated by each rat each day, could be calculated.

Collections of material and counts of tapeworm eggs were made every other day for each rat during the course of the experiment.

For 29 days the young rats had free access to the diet described above while determinations of the numbers of eggs being passed were made every two days. For the last week of this period the weight of the food eaten each day by each rat was determined. One-half of this average daily intake was given to each of six experimental rats (designated in this study as D2, D3, D4, D8, D10, and D12) each day for the next two weeks, while the other six control rats (D5, D6, D7, D13, D14, and D15) were given the same ration but in any desired amount. The weight of the feed eaten by these control animals was determned each day. From the sixth to the ninth week, each experimental animal was given only one-third the average amount of the feed eaten by the control animals during the preceding week. At the ninth week the groups were reversed in treatment; rats D2, D3, D4, D8, D10, and D12 being given food ad libitum and the other six allowed only one-third the average amount eaten by the control animals. This procedure was continued for three weeks when the experiment was terminated.

RESULTS

When the diet was limited in amount as described above, the rats lost weight but maintained a general appearance of health. The egg counts during the periods of partial starvation did not decline appreciably.

Figure 1 illustrates typical weight and egg count variations for two rats, D12 and D15, one in each of the two groups described above. The variations in the total numbers of eggs eliminated by rats of the two groups are graphically represented in figure 2.

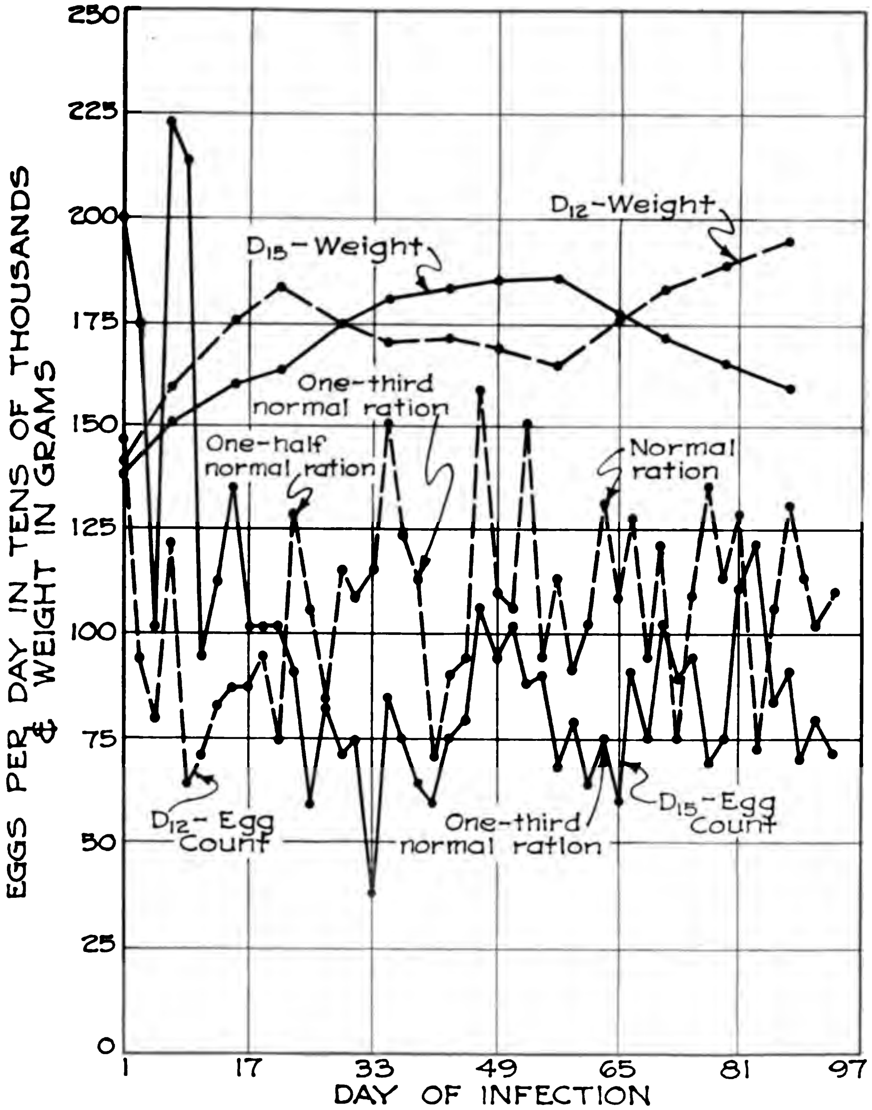

Fig. 1. Variations in body weight and numbers of eggs eliminated for rats D12 and D15 with free and limited food intake.

DISCUSSION

A rather thorough search of the literature has not revealed that the confused flour beetle *Tribolium confusum* has ever before been recorded as a natural or experimental host for the rat tapeworm *Hymenolepis diminuta* (5, 6, 20, 21, 22, 23, 24, 28, 29, 30, 31, 32, 37, 38, 39, 43, 47, 48). It is not surprising, however that the cysticercoid should be able to de-

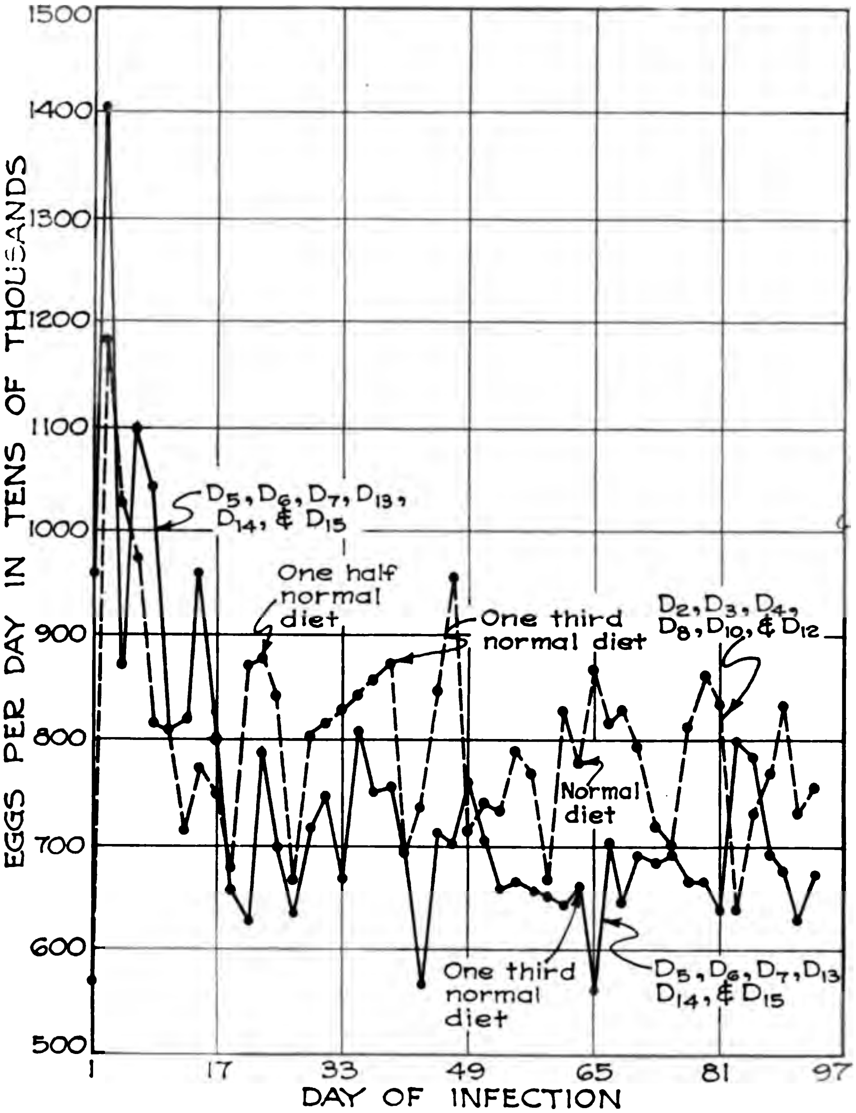

Fig. 2. Comparison of the total number of tapeworm eggs eliminated daily by the group of rats D2, D3, D4, D8, D10 and D12, and the group D5, D6, D7, D13, D14 and D15, with free and limited food intake.

velop in this insect, since it is known to occur in a large number of arthropods and has been reported for the allied species *Tribolium castaneum* (28, 29).

In the present investigation only the adult beetles were used as vectors. Because of the grain-infesting habit of this insect, it seems probable that it might serve as a natural as well as an experimental host. The successful infecting of rats by means of confused flour beetles three weeks after the ingestion of eggs by the insects, suggests that the time required for the development of the cysticercoid in this host is comparable to that found by Joyeux to obtain in other hosts (31).

It is evident from the graphic representations of results that there is a considerable day to day variation in the number of eggs present in the fecal material. This is not unexpected, since varying numbers of segments are separated from the major part of the worm from time to time and the parenchyma partially digested. This day to day variation made it necessary that egg counts should be continued for each rat over a fairly long period of time and that the general trend of the counts should be considered rather than that an attempt should be made to account for each rise or fall.

It may be noted that a high initial production of eggs is characteristic of *Hymenolepis diminuta* infections in the rat. There follows more or less of a leveling off with somewhat lower counts. Although it may be possible that this is due to the development of some immunity on the part of the host, it is questionable whether this can be considered to be the case with such a parasite as *Hymenolepis dimiuuta* which lives in the lumen and is not a tissue dweller. Furthermore, there is no evidence of a continuous development of immunity since the numbers of eggs eliminated did not continue to decline beyond the first week or two of the infection.

CONCLUSIONS

1. The adult flour beetle *Tribolium confusum* may serve as a vector for the rat tapeworm *Hymenolepis diminuta.*
2. The cysticercoids developing in the flour beetle are infective to the rat after approximately three weeks in the intermediate host.
3. Partial starvation of the host by restriction of the food intake to one-third the normal amount does not cause a decrease in the numbers of tapeworm eggs eliminated.
4. A high initial egg production is characteristic of *Hymenolepis diminuta* infections in the rat, followed by a more constant rate of development at a somewhat lower level.

II. DEFICIENCY OF VITAMINS B_1 AND G

A differentiatial effect on intestinal parasites of a diet high in yeast has been reported by Hegner (25) who found that such a diet fed to rats resulted in an increase in the numbers of *Trichomonas muris* and *Hexamitus muris,* but in a decrease in the numbers of *Giardia muris.* Allen (3) reported that chickens harboring *Eimeria tenella* eliminated fewer oocysts for the first five days of the patent period when given a high protein, high vitamin diet than did chickens on a low protein, low vitamin diet. After the fifth day, however, the numbers eliminated by the former group became and remained relatively higher and there was chronic coccidiosis. Becker and Morehouse (11) found that yeast appeared to contain a factor favorable to the growth of *Eimeria miyairii* in the rat, and that the numbers of oocysts were greatly reduced when there was a deciency of vitamins B_1 and G (14). Deficiency of vitamin B_1 alone did not appear to alter the number of oocysts developed, but the vitamin G deficient rats eliminated significantly fewer oocysts than their controls. There was not, however, so great a reduction as in those receiving neither vitamin (12). Later the same authors (13) stated that the factor which promoted growth in coccidia appeared to be linked with the vitamin G complex since wheat germ and other substances furnished it as well as yeast. Liver as a source of the vitamin did not favor the parasite (15). More recently (17) they reported that rats eliminated fewer oocysts when rice polishings, powdered liver or skim milk, in that order, were used as a source of vitamin G, than when the vitamin was supplied by yeast. Vitamin G or some factor associated with it was considered by Becker and Derbyshire to promote growth of coccidia (18, 19).

The effects of dietary modications on helminth parasites also have received attention. Vitamin B_1 was reported by Zimmerman, Vincent and Ackert (49) to be a factor in the resistance of chickens to *Ascaridia lineata*. The worms in the intestines of the chickens on a diet deficient in vitamin B_1, but adequate in other respects, were larger and more numerous than those in chicks of the same age receiving an adequate diet. Ackert and Nolf (1) suggested that yeast may contain a factor favoring the growth of *Ascaridia lineata* in chickens. Ackert (2), however, did not indicate that there is any evidence that this nematode requires vitamin B_1, and, with Beach (8), was not able to demonstrate the presence in yeast of any special growth factor for this parasite.

DEFICIENCY OF VITAMINS B_1 AND G

For the investigation here described, in order to observe the effect upon the tapeworm *Hymenolepis diminuta* of a diet deficient in both vitamins B_1 and G, four young rats (designated as A1, A2, A3, and A4) were parasitized with this cestode in the manner described in Part I; they were maintained on the same control diet and the counts of eggs eliminated per day by each animal were obtained in the same way.

After egg counts had been made for a control period, two of the rats were given a ration similar to that described before, but with yeast omitted and ten per cent more sugar. This diet is comparable to those frequently used for vitamins B_1 and G deficiency. On this diet the rats declined rapidly both in weight and in general appearance of health, and the numbers of worm eggs also declined, disappearing completely from the droppings of one of the hosts. After being restored to the control diet, the rats improved in weight and appearance and the numbers of eliminated eggs mounted. When the other two rats were given the deficient diet, eggs disappeared from the droppings of both and no tapeworms could be found in the intestines at autopsy.

The results obtained for the rat designated as A2 may be taken as an

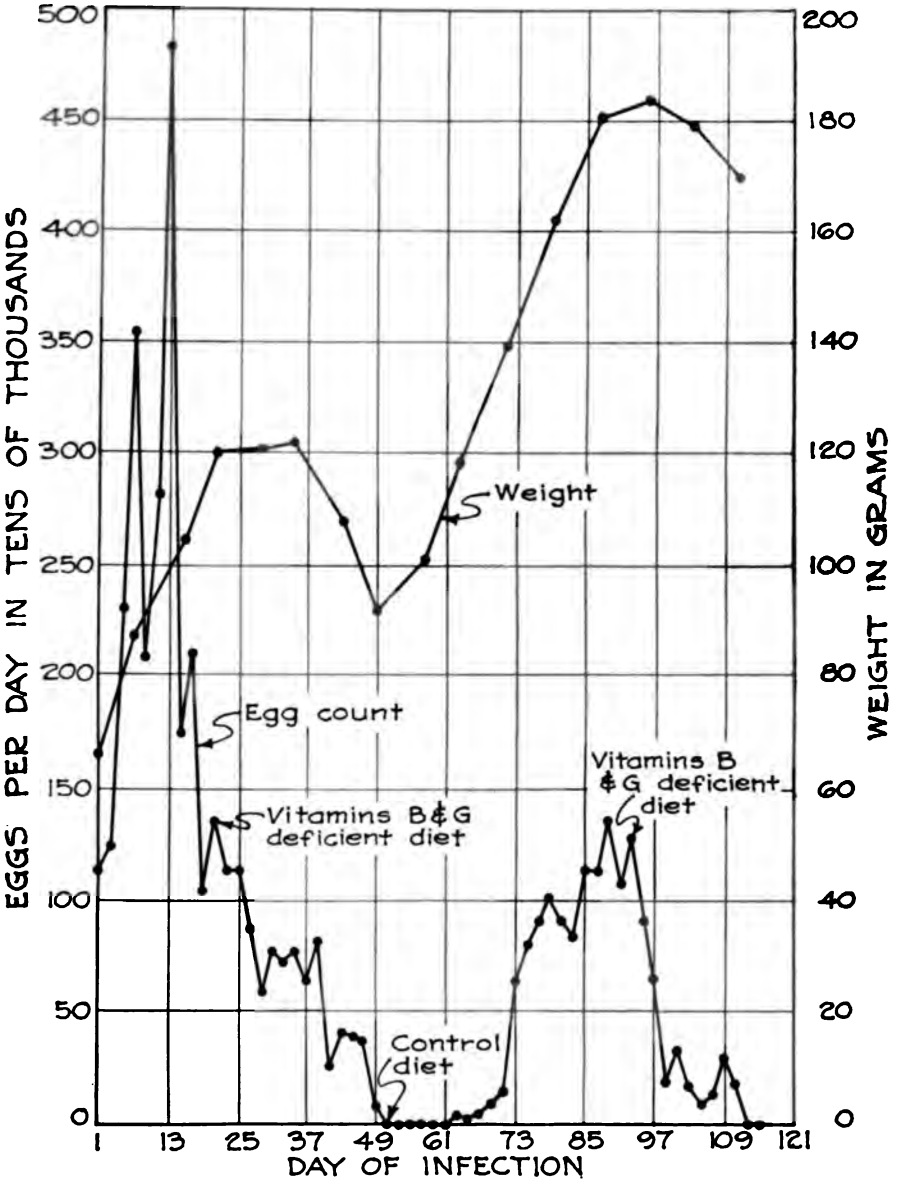

Fig. 3. Variations in the numbers of tapeworm eggs eliminated daily by rat A2 alternately on a control diet and one lacking in vitamins B_1 and G.

example and are represented in figure 3. This host was first maintained on the control diet, then on the experimental ration, restored to the control diet for a time, and then again given the ration lacking in yeast. The variations in the egg counts and in the body weight of the host are indicated in the figure.

Deficiency in Vitamin B_1. In order to observe the effects of a diet deficient only in vitamin B_1, four young rats harboring rat tapeworms were maintained on the control diet described in the foregoing account for a preliminary period of 45 days, during which egg counts were made every two days. Three of the four were then given a ration similar in all respects to the control diet except that the yeast used had been autoclaved for two hours at 120°C. This diet is not adequate for vitamin B_1. Because

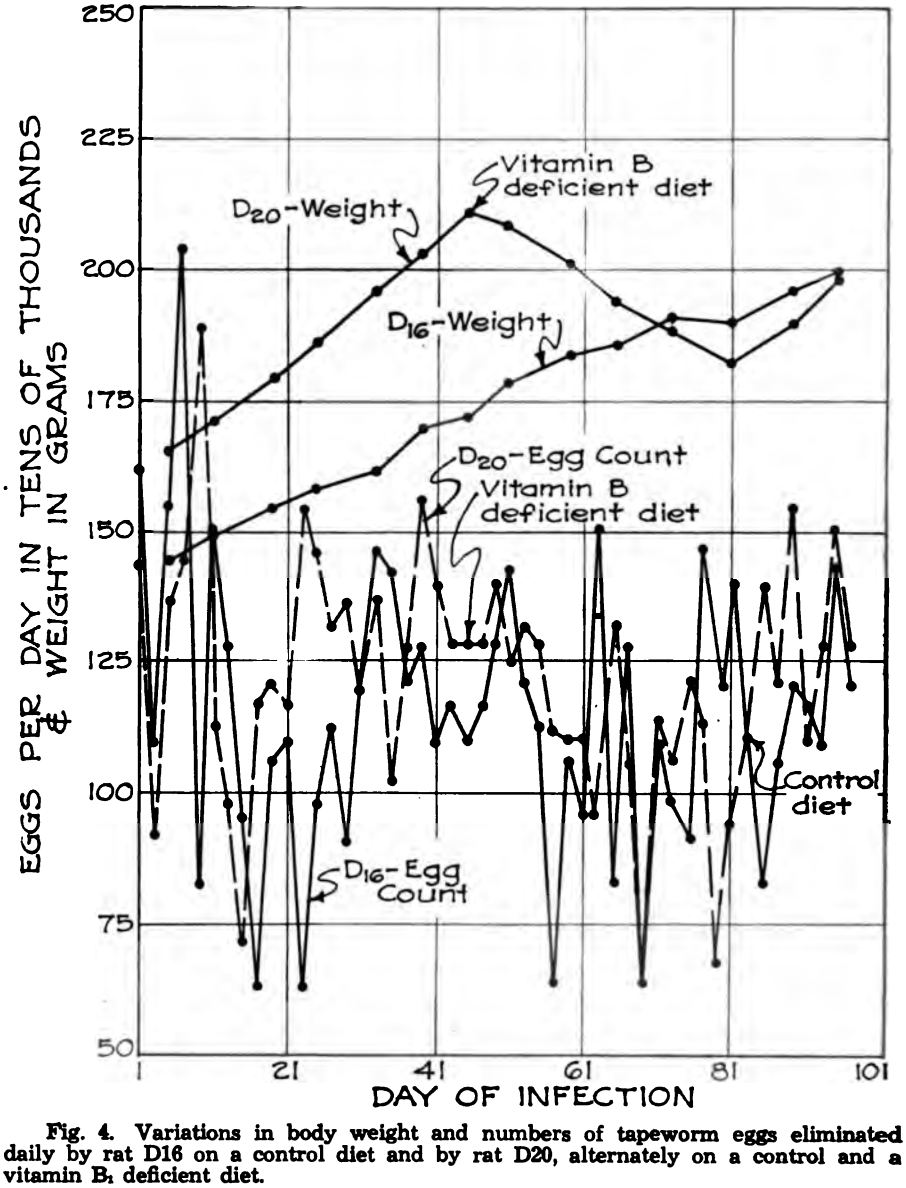

Fig. 4. Variations in body weight and numbers of tapeworm eggs eliminated daily by rat D16 on a control diet and by rat D20, alternately on a control and a vitamin B_1 deficient diet.

the numbers of eggs passed were maintained at the previous level for two of these experimental rats, but declined somewhat in one case, it was considered desirable to try the effect of this diet a second time.

Seven young rats were fed flour beetles exposed to infection with *Hymenolepis diminuta* in the manner described. In the droppings of all but one, eggs were found in from 19 to 23 days. The six young infected rats were fed the control diet containing unautoclaved yeast, and deter-

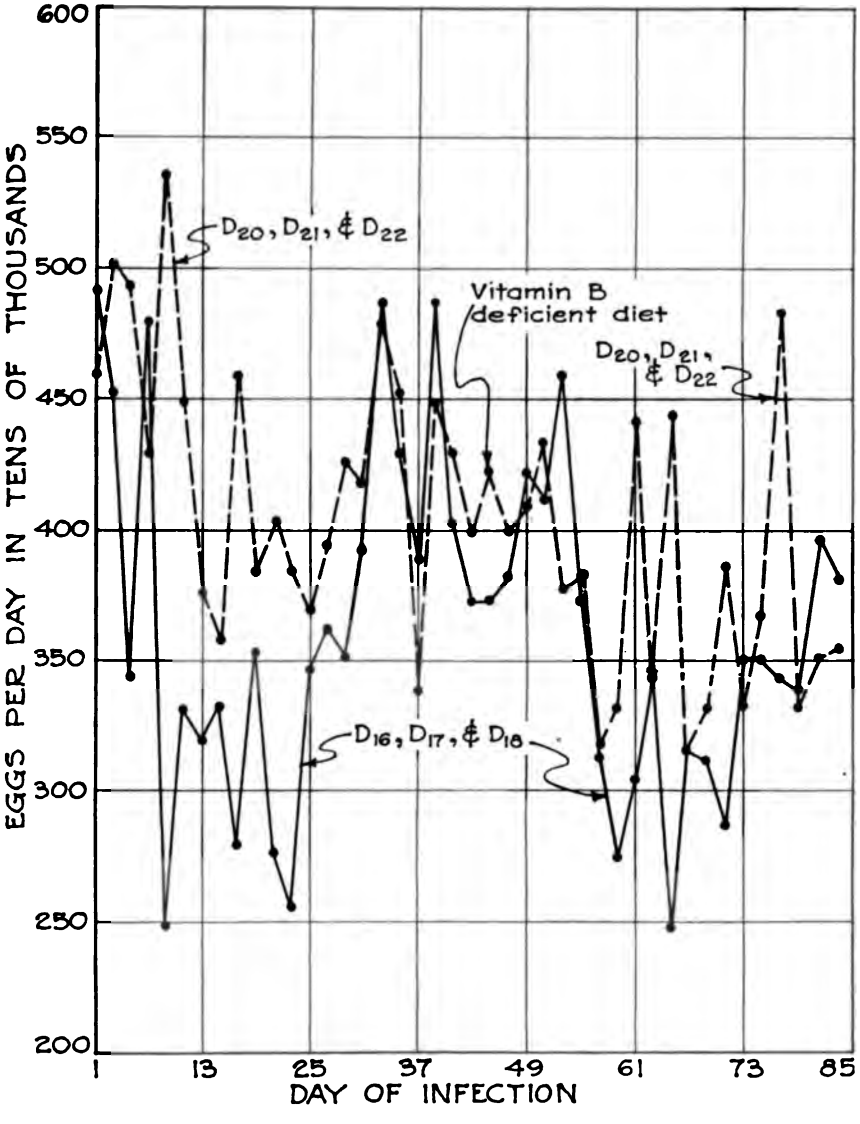

Fig. 5. Comparison of the total numbers of tapeworm eggs eliminated by rats D16, D17 and D18 maintained on a control diet and by rats D20, D21 and D22 maintained alternately on a control and a vitamin B_1 deficient diet.

minations of the numbers of eggs eliminatd were made for a control period of 45 days. At that time the three rats, D20, D21 and D22 were given the diet in which the autoclaved yeast was substituted, whereas the other three, D16, D17 and D18 were continued on the control diet.

The animals on the experimental diet ceased making normal gains and after several weeks fell off sharply in body weight. There was, however, no marked decreased in the numbers of eggs eliminated during this time as compared to those passed during the 45-day control period.

On the eighty-fourth day, one of the experimental animals, D20, was restored to the control diet. This animal made gains in weight, but did not show a notable increase in the numbers of worm eggs passed.

The variations in the weights and numbers of eliminated eggs for the two rats D16 and D20 are shown in figure 4. Rat D16 was maintained continuously on the control diet; rat D20 was shifted to the diet deficient in vitamin B_1 and then again to the control ration.

The total numbers of eggs eliminated by rats D16, D17 and D18 are compared graphically with those passed by rats D20, D21 and D22 in figure 5. Until the forty-fifth day, rats in both groups were given the same diet, the control ration containing unautoclaved yeast. At that time, autoclaved yeast replaced the unautoclaved for one group. This graphic representation is continued only until the eighty-third day, although egg counts were made for an additional two weeks, because after that day the three rats D20, D21 and D22 could not be grouped together, as D20 was again given the control ration.

Effects of a Diet Deficient in Vitamin G. Eight young male rats were fed flour beetles which had been given gravid proglottids of *Hymenolepis diminuta* and all eight became infected. They were maintained on the same control diet, containing unautoclaved yeast, described above. After a month, during which the numbers of eggs being passed daily by each rat were determined, four of the rats, C1, C2, C3 and C4, were transferred to the diet containing no yeast, and consequently deficient in both vitamins B_1 and G. Vitamin B_1 was supplied by the daily administration of four drops of tiki tiki[5] to each experimental rat. This was readily taken from a pipette by those animals whose diet lacked yeast.

The rats on the experimental diet lost weight although they continued to eat fairly well. The skin became coarse; the hair, matted and unkempt. On the one hundred thirty-ninth day of the infection, two of the experimental rats were again given the control diet. These animals regained weight and a general appearance of health and tidiness.

The numbers of eggs passed by rats maintained on the vitamin G deficient diet were greatly decreased. Typical results are presented graphically in figure 6. Rat C1 was fed the control ration for the first month of the infection, transferred to the experimental food for three and a half months and again placed on the control ration for a month. Rat C4 was transferred to the experimental ration for the duration of the experiment

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[5] Obtained from the Bureau of Science, Manila, Philippine Islands.

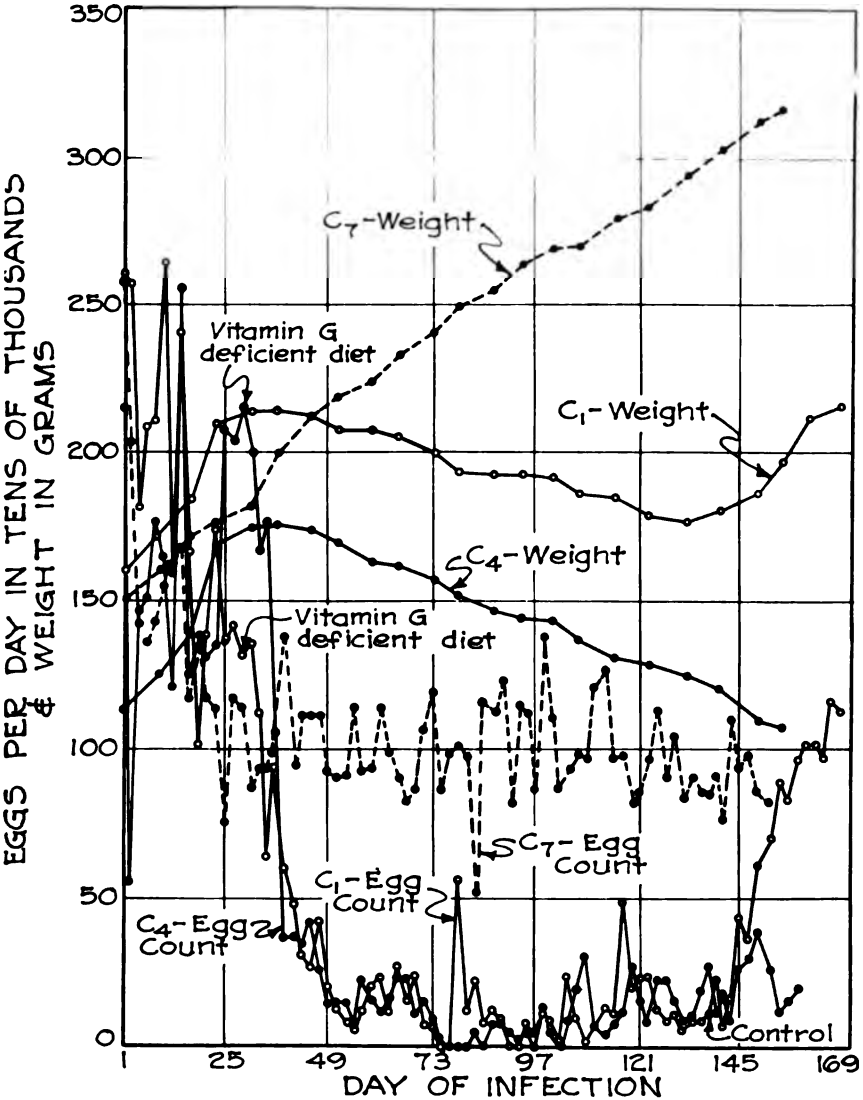

Fig. 6. Variations in body weight and numbers of tapeworm eggs eliminated daily for rats C1, C4 and C7 on vitamin G deficient and control diets.

after being given the control ration for the first month of infection. Rat C7 was maintained for the entire period on the control diet. Both the egg counts and the body weights of the hosts are represented in the figure.

Figure 7 compares graphically the total numbers of eggs passed by rats C1, C2, C3 and C4 which were transferred to the experimental diet, and those eliminated by rats C5, C6, C7, and C8 which continued on the control diet. The figure indicates the course of the infection from the first to the one hundred thirty-ninth day.

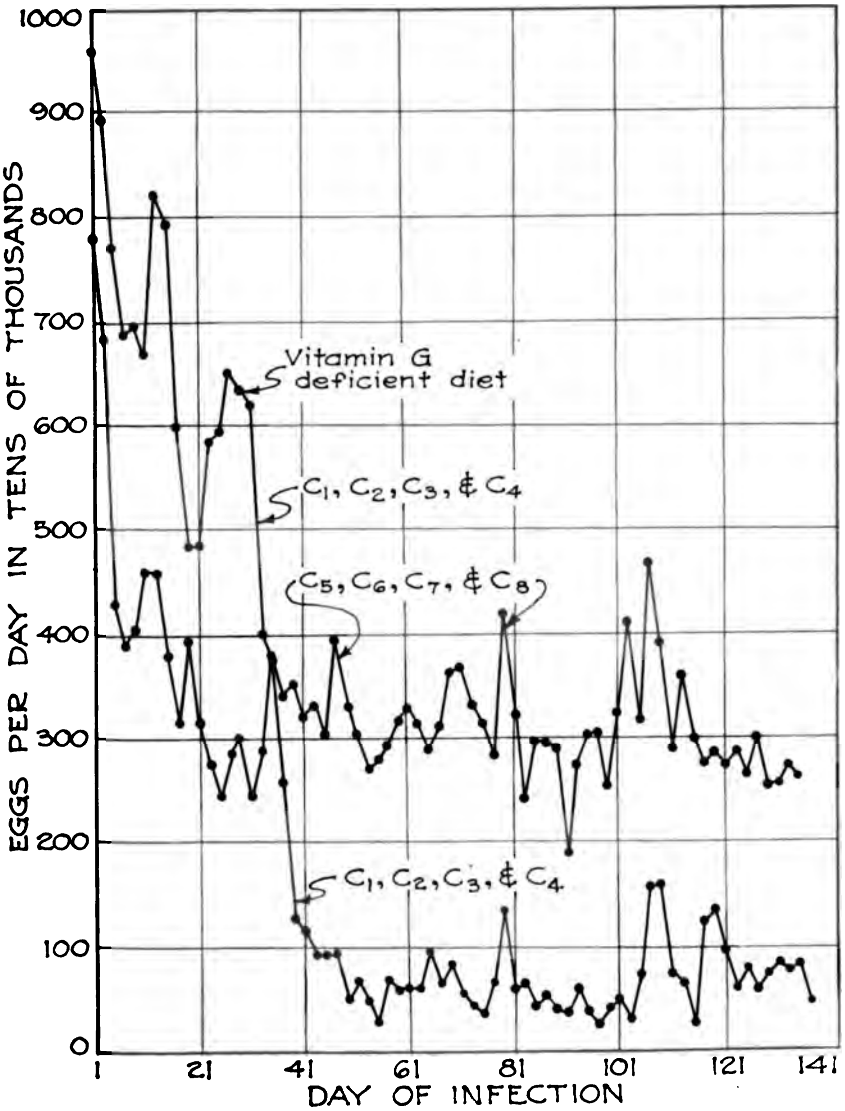

Fig. 7. Comparison of the total numbers of tapeworm eggs eliminated by rats C5, C6, C7 and C8 maintained on the control diet and by rats C1, C2, C3 and C4 given alternately a control and a vitamin G deficient diet.

DISCUSSION

That it is possible to influence the reproductive processes of the rat tapeworm by the diet of the host seems evidenced by the observations described. There appears to be some factor present in yeast which is required for maximum reproductivity of the rat tapeworm. The fact that the production of eggs did not decrease when the yeast had been autoclaved (see figures 4 and 5) suggests that this factor is not the labile vitamin B_1. The decline of reproductive power of the parasite when the host was maintained on a vitamin G deficient diet, however, indicates that it may be associated with the vitamin G complex from some sources. It cannot be stated that this factor is identical with vitamin G.

It has been suggested to the author that the decrease in numbers of eggs eliminated might be merely a secondary result of the decreased amount of fecal material because of less residue from lowered food intake on the part of the host. However, since the rats were continually kept over pans so that all of the pellets passed were collected, and since the determinations were made, not on the basis of numbers of eggs per unit weight of feces, but on numbers of eggs eliminatd over a two-day period, it would seem that all the eggs produced would be accounted for, whether they occurred in a small number of hard pellets or in a larger number of bulky ones.

Furthermore, this suggestion would not explain why egg counts declined on the vitamin G deficient diet which was associated with loss of body weight on the part of the hosts, but were not decreased on the vitamin B_1 deficient diet, also associated with decreased food intake and loss of weight. Nor would it account for the continued collection of large numbers of eggs when the food intake was limited to one-third the usual amount (see Part I).

CONCLUSIONS

1. It is possible to influence the numbers of eggs produced by the rat tapeworm *Hymenolepis diminuta* through modification of the host's diet.
2. A diet deficient in both vitamins B_1 and G is associated with a decrease in egg output.
3. This effect is apparently not due to the labile vitamin B_1, as a diet deficient in this vitamin is not associated with a lowered production of eggs.
4. There appears to be a factor associated with the vitamin G complex which is necessary for normal egg production by the rat tapeworm. A diet lacking in vitamin G does not furnish this factor. It is not maintained that the factor is identical wtih vitamin G.
5. It is suggested that the effects of the various diets are probably produced directly upon the parasite rather than indirectly through effects on the hosts, since certain diets detrimental to the hosts are not associated with lowered egg production on the part of the parasite, whereas other diets appear to be deleterious to both host and parasite.

III. MILK, SOY BEAN OIL MEAL, WHEAT MIDDLINGS

The maintenance of rats harboring *Giardia muris, Trichomonas muris* and *Hexamitus muris* upon an exclusive milk diet was reported by Hegner (25) to be associated with a decrease in the incidence of all three forms. Ratcliffe (41) found a reduction in the numbers of trichomonads in the ceca of rats fed a diet high in protein in the form of casein. Ratcliffe noted also (42) that numbers of *Trichomonas hominis* and *Pentatrichomonas ardin delteili* became lighter when the host rats were given a diet which increased intestinal acidity and still lighter on a high protein diet containing 56 per cent unpurified casein. The organisms were not eliminated, however, by the use of this diet. In rats and in monkeys, Kessel (33) reported that an exclusive milk diet or neutral diets with lactic acid or large amounts of lactose added, produced a lowering of the pH of the colon contents and a decrease in the amoebae and trichomonads present. Kessel and K'E-Kang (34) fed an exclusive raw milk diet to monkeys and to children and observed that during that period there was almost always a reduction in the numbers of intestinal amoebae, and in some cases the intestine was cleared of *Endamoebae dysenteriae* and other forms. Infections of *Balantidium coli* from the pig were established in rats by Schumaker (45). Within a week the infection was greatly reduced or eliminated from rats on a diet of whole milk. Diets high in casein prevented infection and eliminated heavy infections within eight days, but a high carbohydrate diet containing no casein was favorable for the development of the parasite.

Beach and Davis (7) found that chicks were given an appreciable protection against the effects of coccidiosis by being fed mash containing 40 per cent dry skim milk and 20 per cent lactose. The effect was attributed by the authors to the greater acidity of the cecal contents. Their conclusions were drawn only from observations of the birds, and no counts of oocysts were made.

Becker and Morehouse (10) did not find the addition of skim milk or of lactose to the diet of rats to exert any restraining influence upon the numerical increase of *Eimeria miyairii,* but when the diet was high in casein, the numbers of oocysts were somewhat reduced (14). Rats eliminated fewer oöcysts when skim milk was used as a source of vitamin G, than when the vitamin was supplied by yeast (17).

Certain constituents of diets for chicks, particularly skim milk, buttermilk and wheat middlings in certain amounts or combinations, were reported by Becker (16) to increase the severity of *Eimeria tenella* infections in chicks. Becker and Derbyshire (18) found no correlation between the bulkiness of the ration and the number of oocysts of *Eimeria miyairii* eliminated by rats, nor between the growth of host and parasite development. They found the ratio of coccidium-growth-promoting substance in soy bean oil meal as compared to yeast to be 0.51; and in wheat flour middlings, 2.65 (19).

Reports of relations of diets to cestode parasites are not numerous. Becker (9) gave only whole milk (plus copper sulfate and iron chloride for seven of the hosts) to 15 rats, all passing eggs of *Hymenolepis nana,* and kept on screen to prevent coprophagy. After five and one-half days, no eggs could be found by smear or flotation, nor were eggs passed subsequently during the 30 days that the rats were on a milk diet. After this time the rats were again given a grain diet, and eggs reappeared in the feces of 11. No tapeworms were found in the others. Milk apparently produced a cessation of egg production by the parasite, but not death.

THE EFFECTS OF AN EXCLUSIVE RAW MILK DIET

In the investigation here reported, six young rats maintained on Steenbock's growing ration (yellow corn meal, 71 per cent; linseed oil

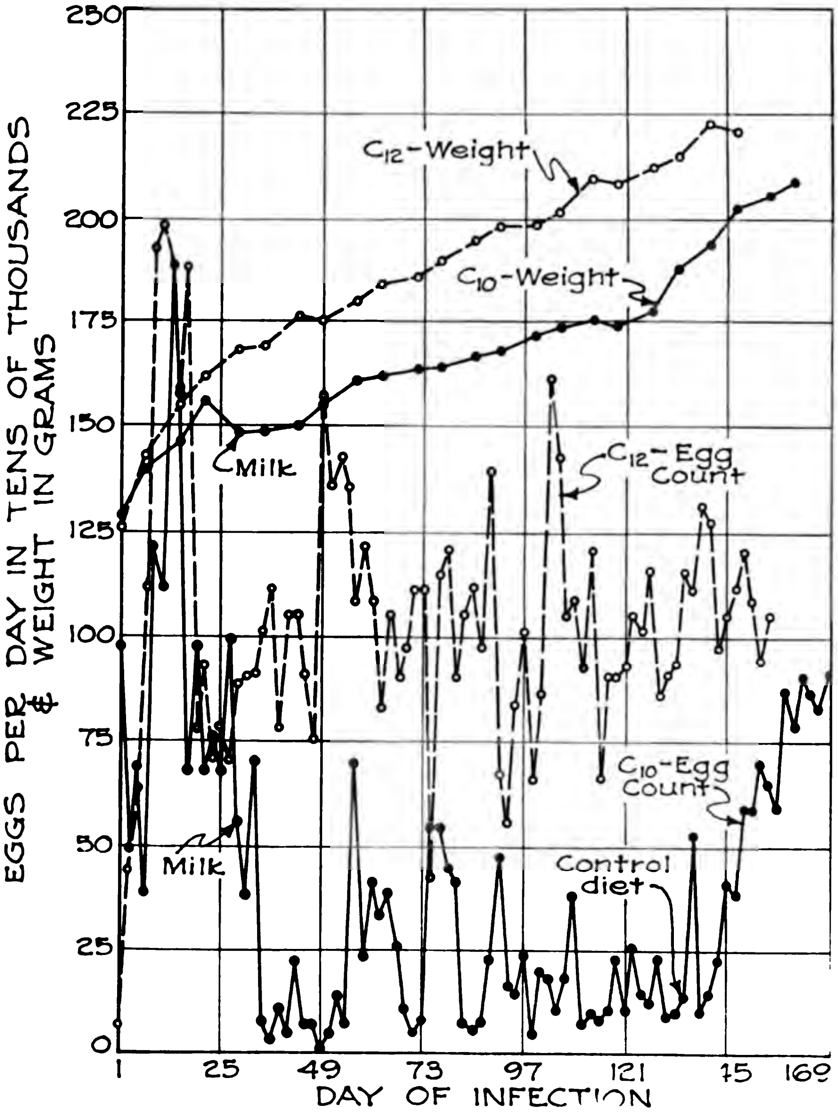

Fig. 8. Variations in body weight and numbers of tapeworm eggs eliminated daily for rat C12, on Steenbock's ration, and rat C10, alternately on Steenbock's ration and milk.

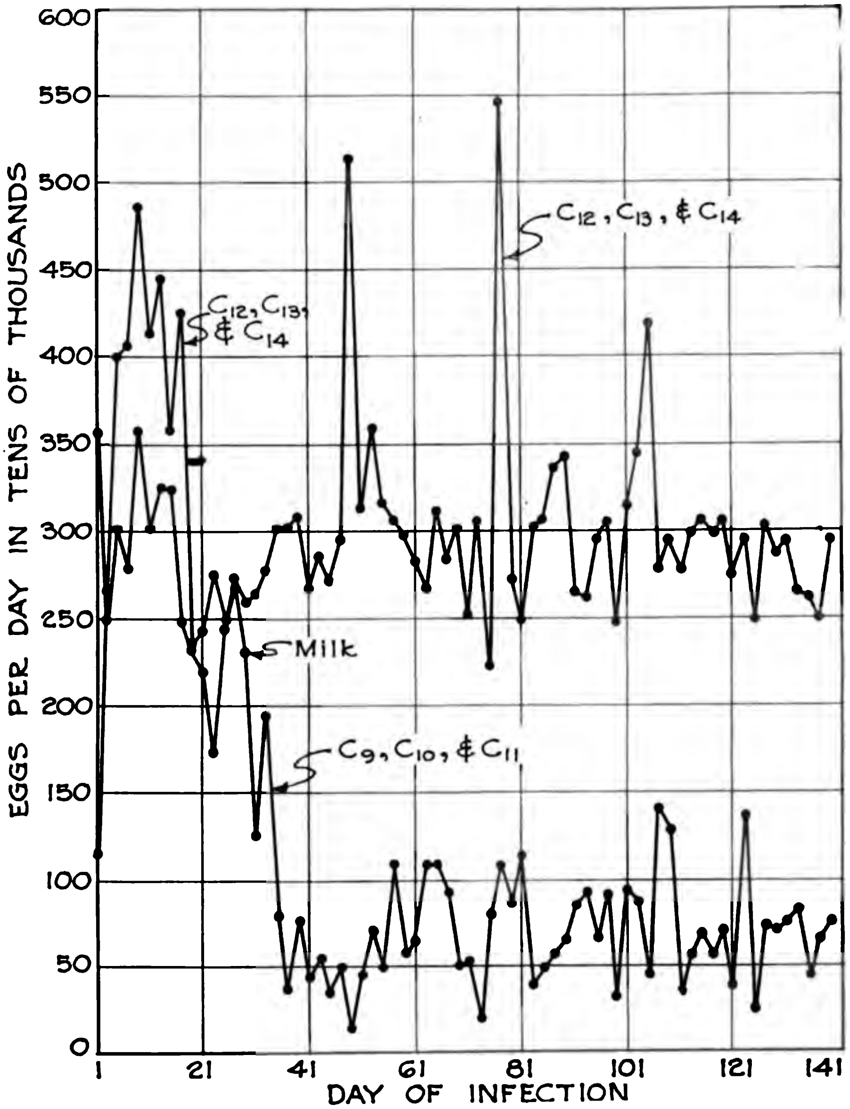

Fig. 9. Comparison of the total numbers of tapeworm eggs eliminated daily by rats C12, C13 and C14 on Steenbock's ration and by rats C9, C10 and C11, alternately on Steenbock's ration and milk.

meal, 16 per cent; ground alfalfa, 2 per cent; sodium chloride, 0.5 per cent; calcium carbonate, 0.5 per cent; dried skim milk, 10 per cent) were fed infected flour beetles[6] and all began to pass eggs of *Hymenolepis diminuta* in from 19 to 25 days. The numbers eliminated each day by each rat were determined. On the thirtieth day after the first occurrence

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[6] According to the method described in Part I.

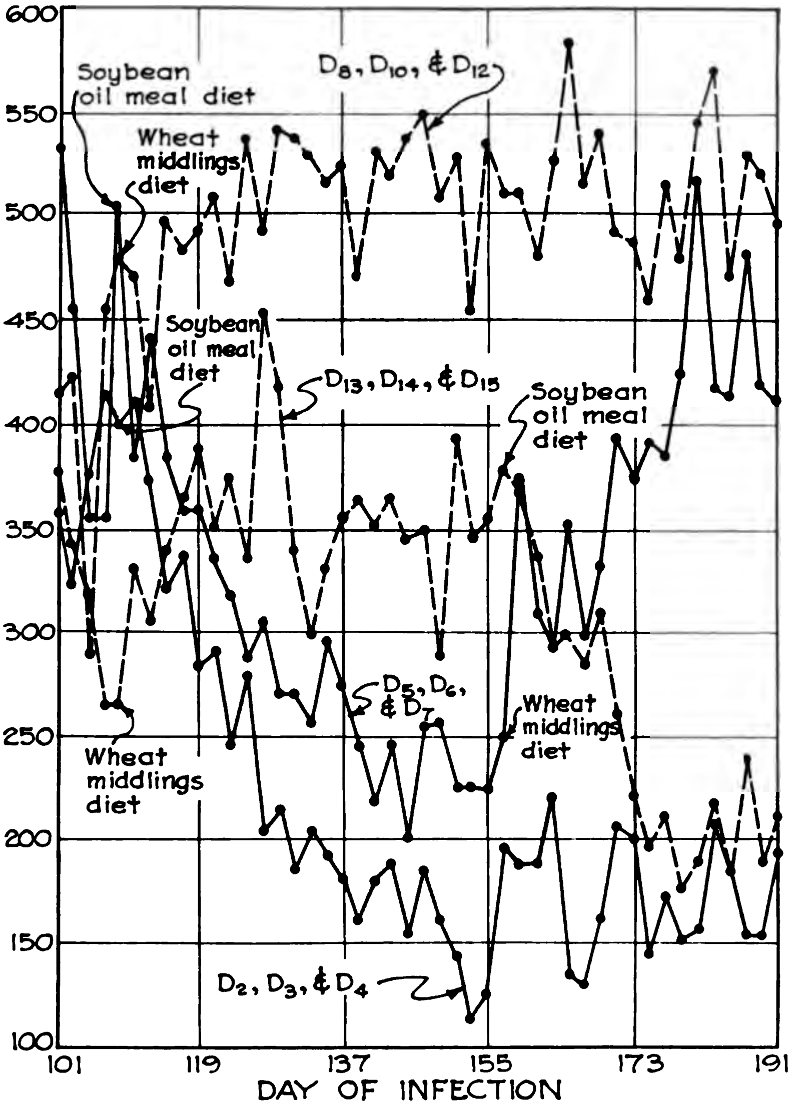

Fig. 10. Total numbers of tapeworm eggs eliminated daily by the group of rats D2, D3 and D4; the Group D5, D6 and D7; the group D8, D10 and D12, and the group D13, D14 and 15 maintained on diets containing soy bean oil meal or wheat middlings.

of eggs in the pellets, three of the rats, C9, C10 and C11, were taken off the growing ration and given only whole raw milk. The other three rats were maintained on the growing ration as before. Egg counts were made over a period of four months. On the one hundred thirty-fifth day of the infection, one rat, C10, was restored to the growing ration, but rats C9 and C11 were continued on milk. The egg counts for C10, typical of the results for the experimental rats, and for C12, representative of the controls, are shown graphically in figure 8. The weight variations of the two rats are indicated in the same figure.

Figure 9 shows a graphic comparison of total numbers of eggs eliminated each day by the three experimental rats, C9, C10 and C11, with those passed by the three controls, C12, C13 and C14, from the first to the one hundred thirty-fifth day. The three experimental rats cannot be grouped subsequent to that day, as they no longer all received the same diet.

COMPARISON OF THE EFFECTS OF SOY BEAN OIL MEAL AND WHEAT MIDDLINGS IN THE DIET

Twelve young rats harboring rat tapeworms were divided into two groups of six rats each. The rats in one group were given a modification of the control diet in which 10 per cent soy bean oil meal was substituted for the 10 per cent yeast; the other, a diet in which 30 per cent wheat middlings was substituted for the 10 per cent yeast and 20 per cent of the sugar.

After 46 days on these rations, three of the rats in each group were shifted to the alternate diet; the other three were allowed to continue as before.

The rats continued to gain on both rations at approximately the same rate. The numbers of eggs eliminated, however, were appreciably higher when the modified diet included wheat middlings than when soy bean oil meal was substituted, as may be seen in figure 10. This figure represents graphically the total numbers of eggs eliminated by rats D2, D3 and D4 which were maintained on the soy bean oil meal diet for the course of the experiment, and the numbers passed by rats D5, D6 and D7 which were first maintained on the soy bean oil meal ration and then shifted to that containing wheat middlings. Figure 3 also shows the total counts for D8, D10 and D12 on the wheat middlings diet, and for D13, D14 and D15 first on wheat middlings and then on soy bean oil meal. In this way comparisons may be made between the results obtained for those rats on each of the diets for the entire period of observation, and for those transferred from one diet to the other.

CONCLUSIONS

It has been previously suggested that there is some factor present in yeast and associated with the vitamin G complex which is necessary for maximum reproductivity of the rat tapeworm. (See Part II of this series.) Raw milk appears to furnish an insufficient amount of this factor to keep the worm at its maximum reproductive level; wheat middlings seem to furnish more than does soy bean oil meal, although this latter supplement does not lack it entirely.

IV. OBSERVATIONS ON WORM BURDENS

In the previous papers of this series[7], procedures were described in which the rat hosts were maintained on various diets (Steenbock's growing ration, raw milk, diets adequate and deficient in both vitamins B_1 and G and in each of these vitamins separately, and diets containing soy bean oil meal or wheat middlings), and the effects on the reproductivity of the cestode parasite *Hymenolepsis diminuta* were noted. In the planning of these experiments it was considered necessary to have a control period for each rat so that during the experimental period its elimination of eggs could be compared to its previous record in that respect. The egg counts for one rat could not be directly compared with those from another since there was no way of assuring that they should be infected with the same number of worms.

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[7] Parts I, II, and III.

When, at the conclusion of any of the investigations referred to above, the rats were killed with illuminating gas and the intestines opened, the tapeworms present were removed and counted. The parasites were allowed to relax for one hour in cold water and then were measured.

RELATION OF THE WORM BURDEN TO THE NUMBERS OF EGGS ELIMINATED

For those rats (numbering 26 altogether) in the various experiments which were maintained on the ration designated as the control diet, for the first month of infection, it was possible at autopsy to obtain data on the relationship of the egg counts to the numbers of tapeworms harbored. There was revealed no direct correlation between the numbers of eggs found in the droppings and the worm burden, but rather it appeared that the number of eggs produced by a worm was influenced to some extent by the number of parasites present, so that a rat harboring a considerable number of worms might pass no more eggs than one harvoring only a few or even a single parasite, as shown in table 1. In this table, those rats found to have harbored the same number of parasites are grouped, and the average number of eggs eliminated per day by each rat during the first month of infection noted, together with the mean for each group. The average number of eggs produced per day for each worm was calculated and also appears in the table. During the period involved, all of these rats had similar adequate diets.

These results are represented graphically in figure 11 which shows the average number of eggs passed per day per rat for each group harboring different numbers of parasites, and in figure 12, which represents the average number of eggs produced per day per worm for the same groups.

RELATION OF THE WORM BURDEN TO THE LENGTHS OF WORMS HARBORED

In table 2, all of the 32 rats used in the investigations previously described, regardless of diet before autopsy, are arranged in groups in an ascending series according to the numbers of tapeworms harbored. The average length of the parasites in each host is recorded, together with the diet which that host had been receiving prior to its death. The "control" diet is the adequate, complete food mixture described in Part I and designated as such throughout this series of papers; the diet indicated as "Steenbock's" is Steenbock's growing ration (Part III); "milk"

TABLE 1· *Mean numbers of tapeworm eggs eliminated per day per rat and per day per worm for rats on an adequate control diet, but harboring different numbers of parasites*

Number of worms harbored	Rat	Average number of eggs (10^3) eliminated per day for first month of infection	Average number of eggs (10^3) per worm per day
1.	D8	1777	1777
1.	C7	1439	1439
1.	D19	1246	1246
1.	D3	1233	1233
1.	D2	978	978
1.	D12	940	940
1.	C5	430	430
		(Mean: 1149)	(Mean: 1149)
2.	D4	1478	739
2.	D5	1460	730
2.	D15	1378	689
2.	D16	1284	642
2.	D13	994	497
		(Mean: 1319)	(Mean: 659)
3.	D22	1359	453
3.	D17	782	294
		(Mean: 1070)	(Mean: 373)
4.	D6	1684	421
4.	C8	1520	380
		(Mean: 1602)	(Mean: 400)
5.	D21	1735	347
6.	C1	1848	308
7.	D10	1950	280
7.	D7	1715	245
		(Mean: 1832)	(Mean: 262)
8.	D14	1808	226
10.	C3	1790	179
11.	C4	1595	145
12.	D18	1404	117
14.	C2	1694	121
42.	C6	672	16

refers to an exclusive whole raw milk diet; "soy bean oil meal" and "wheat middlings", to the diets including these supplements (Part III).

DISCUSSION

For several reasons, it seems probable that all the worms harbored by any one rat were received at the same time. The infected beetles were kept in glass jars with tight screw caps to prevent their escape, and the immediate dropping of the rat pellets through the bottoms of the cages into the trisodium phosphate solution in the pans makes it unlikely that any other insects would have been accidentally infected. It might be

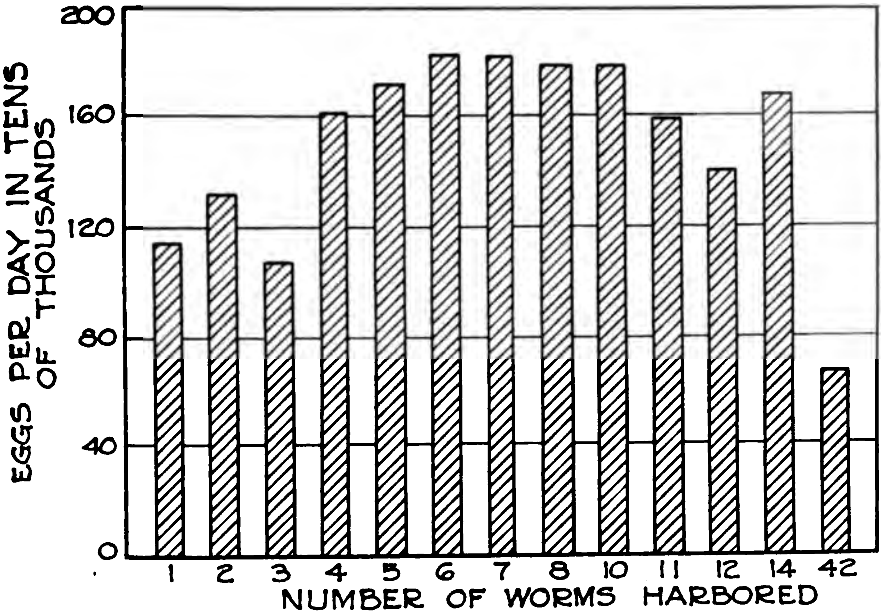

Fig. 11. Mean numbers of tapeworm eggs eliminated per day by rats harboring various numbers of worms.

noted in this connection that from time to time uninfected rats were kept for considerable periods in the same laboratory and none of them were ever found to have become hosts for the rat tapeworm, although the droppings were examined periodically. Moreover, Palais (40) reports his inability to infect rats with additional rat tapeworms once an infection was established. On the other hand, no evidence was ever seen in the pans of spontaneous loss of a worm. Series of proglottids were sometimes

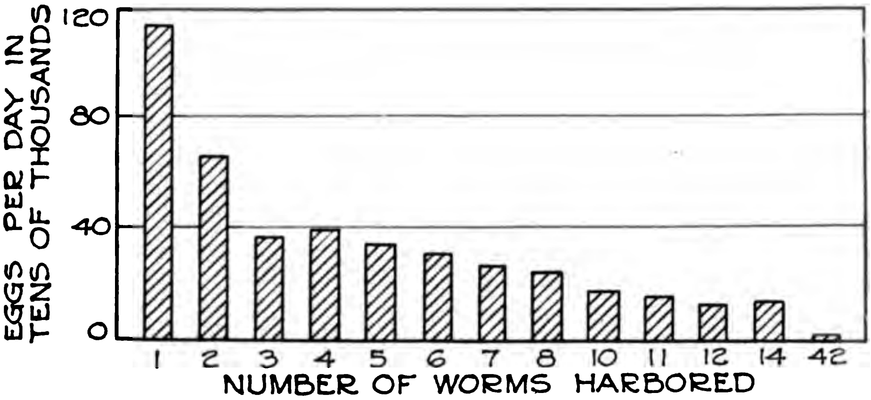

Fig. 12. Mean numbers of eggs produced per worm per day by worms grouped according to the numbers of worms present.

present in the droppings, but these were always gravid proglottids from the posterior portion of the worm. It seems, therefore, that during the course of any investigation, there was no change in the number of worms present in any one host and that the variations in the egg counts represent actual variations in the numbers of eggs being developed rather than changes in the worm burden of the host.

It was not possible to compare results on a per worm basis, since the numbers of eggs produced per worm are not the same for different severities of infection (see figure 12). Only the general trend of one egg count curve may legitimately be compared with that of another.

Reference to table 1 and to figure 11 will show that there was little difference in the average numbers of eggs eliminated by rats harboring one, two or three tapeworms, and rats harboring from four to fourteen worms eliminated very nearly the same average numbers. A single helminth parasite of this type harbored in an intestine would seem to have optimum conditions for reproduction and the numbers of eggs developed by any worm decrease fairly regularly as the worm burden increases (see figure 12).

Table 2 illustrates the tendency for the parasites to be smaller as the worm burden increases. When as many as 42 worms were harbored, some remained extremely small, and examination by microscope did not reveal any eggs whatever. Young and mature proglottids were present but no gravid segments, and the width of the worms was less than the width of corresponding portions of the larger worms. The differences in size of parasites as well as differences in the numbers of eggs produced in infections of differing severity might conceivably be due to competition for food or to crowding. If the decreased amount of food available for each parasite were the determining factor, it would be expected that limiting the food intake of the host would have shown similar results. Yet, reducing the amount of food ingested by the host to one-half or even to one-third the normal amount produced no apparent decrease in reproductive rate or power (See Part I), whereas worms dwelling in an intestine with one other worm produced only 57 per cent (See table 1) as many eggs as those worms which were the sole residents.

If the results observed be ascribed to crowding, the effects might have been produced by the actual pressure and lack of room for growth. Indeed, when many worms are harbored the intestine gives the impression of being so full of parasites as to be nearly obstructed. The decreased opportunity for absorption when many worms are crowded together may be a factor as may also a detrimental effect of the greater amount of excretory products present from the many parasites.

A decrease in reproductive power associated with an increased parasite population has been noted by other investigators. Hill (27) reports a decrease in the number of eggs produced per hookworm as the number of worms harbored by the human host increased. In the case of the dog hookworm, Sarles (44) found a smaller egg production per worm in

TABLE 2. *Mean lengths of tapeworms found in rats harboring different numbers of worms*

Number worms Present	Rat	Average length of tapeworms (cm.)	Diet of host before death
1	D20	92	Control
1	D21	91	Soy bean oil meal
1	C5	89	Control
1	D8	88	Wheat middlings
1	C7	78	Control
1	D12	76	Wheat middlings
1	D3	71	Soy bean oil meal
		(Mean: 83.6)	
2	D16	86.5	Control
2	C10	74	Steenbock's
2	D15	71.5	Soy bean oil meal
2	D13	69.5	Soy bean oil meal
2	D4	58	Soy bean oil meal
2	D5	53	Wheat middlings
		(Mean: 68.8)	
3	D17	93	Control
3	D22	82	Vitamin B deficient
3	C11	64	Milk
3	C14	59	Steenbock's
		(Mean: 74.5)	
4	D6	54.5	Wheat middlings
4	C8	51.5	Control
		(Mean: 53)	
5	C13	57	Steenbock's
5	D21	56	Vitamin B deficient
		(Mean: 56.5)	
6	C1	43	Control
7	D10	47	Wheat middlings
7	D7	44	Wheat middlings
7	C9	41	Milk
		(Mean: 44)	
8	D14	36	Soy bean oil meal
9	C12	38	Steenbock's
10	C3	37.5	Vitamin G deficient
11	C4	39	Vitamin G deficient
12	D18	31.3	Control
14	C2	38	Control
42	C6	32	Control

heavy infestations than in light ones, but the difference of size of worms in light and heavy infestations was slight as compared to the variation between those of similar severity of infection in different dogs. Andrews (4) reports that, as the population of the sheep nematode *Cooperia curticei* increased, the egg production per female worm was found to decrease.

In *Hymenolepis fraterna* infections in rats and mice, Shorb (46) found that after the eighth day the larger worms were present in the hosts having fewer parasites and he suggests that, as the worms grow, crowding becomes a factor in retarding growth.

CONCLUSIONS

1. There is no direct relationship between the number of rat tapeworms harbored and the number of eggs eliminated daily by the rat host.
2. There is an inverse relationship between the number of worms harbored by a rat and the average number of eggs produced by each worm, a fact possibly attributable to crowding.
3. There is a tendency for tapeworms to be shorter when present in large numbers within a host than when present singly.
4. When large numbers of worms are harbored, some of the worms may fail to develop gravid proglottids and to produce eggs.
5. Spontaneous loss of the tapeworm *Hymenolepis diminuta* does not occur readily from the rat.

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[1] Conclusions for Parts I, II, and III will be found on pages 131, 139 and 144 respectively.

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